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Anti-obesity Effects of Fermented Ginseng Root and Berry in Mice Fed a High-Fat Diet

고지방 식이 섭취 마우스에서의 발효한 인삼 뿌리와 열매의 항비만 효과

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by

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Abstract

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Ginseng has been used as a precious remedy for thousands of years. A review of previous *in vitro* and *in vivo* studies reveals that ginseng and ginsenosides, the main bioactive components of ginseng, can increase energy expenditure by stimulating the AMP-activated protein kinase (AMPK) pathway and reduce excess energy consumption via suppressing the over intake of food and retarding pancreatic lipase. The representative ginsenosides in ginseng root are the protopanaxadiol (PPD)-type while that in ginseng berry are the protopanaxatriol (PPT)-type. The root and the berry may show a different pattern of activity due to the distinct ginsenoside profiles. Now that the deglycosylated forms of ginsenoside are more easily absorbed and exert more potent bioactivities, it is necessary to transform ginsenosides before oral ingestion. Retarding the digestion and absorption of fats in the intestine reduces energy harvest, which helps to prevent and improve obesity. The present research is aimed to screen various strains of *A. niger* and *A. orzyae* in order to transform ginsenosides in ginseng root and ginseng berry, and compare the anti-obesity effects between the root

and the berry saponin in the aspects of inhibition on the activity of pancreatic lipase, and regulation of body weight and lipid metabolism in obese mice induced with high-fat diet. The results show that *A. niger* is more apt to transform the PPD-type ginsenoside to compound K (cK) while *A. orzyae* is more apt to transform the PPT-type ginsenoside to Rh1. Ginseng root and berry fermented with mycotoxin non-producing *A. niger* FMB S494 and *A. oryzae* FMB S247 contains abundant cK and Rh1, respectively. Assay of pancreatic lipase activity shows that the PPD-type ginsenosides possess more potent inhibitory effect than the PPT-type, and that transformation dramatically enhances the inhibitory effects of the root saponin and the berry saponin. Furthermore, HFD-fed mice orally administered with the root saponin have significantly higher levels of triglyceride in their feces. It therefore can be concluded that the root saponin exerts more potent inhibitory effect on the activity of pancreatic lipase than the berry saponin both *in vitro* and *in vivo*. Animal study shows that both the saponins significantly suppress body weight gain and improve hypercholesterolemia and fatty liver while only the root saponin significantly attenuates hyperglycemia and insulin resistance. Both the root saponin and the berry saponin have a beneficial effect on HFD-induced obesity. Compared to the berry saponin, the root saponin exhibits more potent anti-hyperglycemic and anti-obesity effect. However, only the berry saponin significantly inhibits mRNA expression of inflammatory markers

such as IL-1β and IL-6 in adipose tissue. Now that cK and Rh1 are respectively the absorptive forms of the PPD-type and the PPT-type ginsenosides, whether they are responsible for the anti-obesity effects of the root saponin and the berry saponin, respectively, needs to be confirmed. Additional animal study shows that both the root saponin and cK significantly reduce excess calorie consumption, body weight gain, food efficiency, fat deposition, and down-regulate the expression of gene *Fas* in the adipose tissue. It therefore can be concluded that cK is responsible for the anti-obesity activities of fermented ginseng root. The berry saponin also slightly reduces body weight gain, food efficiency, and down-regulates the expression of gene *Fas* in the adipose tissue while ginsenoside Rh1 only reduces fat deposition, which indicates that there might be other ginsenosides or other active compounds responsible for the anti-obesity effect of fermented ginseng berry. In conclusion, cK and the root saponin respectively show more potent anti-obesity effects than Rh1 and the berry saponin considering their inhibitory effects on the activity of pancreatic lipase, excess food intake, and body weight gain.

Keywords: ginseng root; ginseng berry; pancreatic lipase; high-fat diet, obesity

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List of Abbreviations

ACAT: acyl-CoA: cholesterol acyltransferase

ACC: acetyl-CoA carboxylase

AdipoR: adiponectin receptor

ALT: alanine transaminase

AMPK: adenosine monophosphate-activated protein kinase

ANOVA: analysis of variance

AST: aspartate transaminase

ATGL: adipose triglyceride lipase

BMI: body mass index

CD: cluster of differentiation

C/EBP: CCAAT-enhancer-binding protein

cK: compound K

CoA: concanavalin A

COX-2: cyclooxygenase 2

CREB: cAMP response element-binding protein

CYP7A1: Cholesterol 7 alpha-hydroxylase

CYP8B1: sterol 12 alpha-hydroxylase 1

DAD: diode array detector

DGAT: diacylglycerol acyltransferase

ELISA: enzyme linked immunosorbent assay

FABP4: Fatty acid binding protein 4

FAS: fatty acid synthase

FER: food effect ratio

FGF2: basic fibroblast growth factor 2

FGR: fermented ginseng berry

FGR: fermented ginseng root

G6Pase: glucose 6-phosphatase

GLUT4: Glucose transporter type 4

GR: ginseng berry

GR: ginseng root

HDL: high density lipoprotein

HFD: high-fat diet

HMGCR: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase

HOMA-IR: homeostatic model assessment-insulin resistance

HPLC: high performance liquid chromatography

HSL: hormone sensitive lipase

IFN: interferon

IL: interleukin

iNOS: inducible nitric oxide synthase

IRS: insulin receptor substrate

LDL-R: low density lipoprotein receptor

LFD: low-fat diet

LPL: lipoprotein lipase

LPS: lipopolysaccharide

LRP1: low density lipoprotein receptor-related protein 1

MCP: monocyte chemoattractant protein

MHC: major histocompatibility complex

MMP: matrix metalloproteinases

MRP2: multidrug resistance-associated protein

NAFLD: non-alcoholic fatty liver disease

NEFA: non-esterified fatty acid

NK: natural killer

NO: nitric oxide

NPY: neuropeptide Y

NRF-1: nuclear respiratory factor 1

PBS: phosphate buffered saline

PDA: potato dextrose agar

PEPCK: phosphoenolpyruvate carboxykinase

PGC-1α: PPAR-γ coactivator-1α

PHA: phytohaemagglutinin

PPAR: Peroxisome proliferator-activated receptor

PPD: protopanaxadiol

PPT: protopanaxatriol

PTP1B: protein-tyrosine phosphatase 1B

RT-PCR: Real-time polymerase chain reaction

SGLT1: sodium-dependent glucose transporter 1

SOCS3: suppressor of cytokine signaling 3

TC: total cholesterol

TG: triglyceride

TIMP: tissue inhibitor of metalloproteinase

TLC: thin-layer chromatography

TNF: tumor necrosis factor

VEGF-A: vascular endothelial growth factor A

VLDL: very-low-density lipoprotein

Chapter 1 Introduction

1.1 Ginseng and its active components

Panax ginseng (C.A. Meyer, family Araliaceae) has long been used as a kind of novel medicinal herb in Korea, China, Japan and other countries. It was first recorded in *the Herbal Classic of the Divine Plowman*, the oldest comprehensive materia medica, about 2000 years ago [1]. Panax ginseng is a kind of perennial herb with fleshy roots, widely distributed in Eastern Asia, mostly like Korea, northeast China and east Russia, typically in cool climates at a latitude between N 30° and N 48°. The English word "ginseng" derives from the Chinese term "*rénshēn*"; "rén" means "person" and "*shēn*" means "plant root". The English pronunciation originates from a Cantonese reading. Commercial ginseng is sold in over 35 countries with sales exceeding $2.1 billion in 2013, of which half came from South Korea [2].

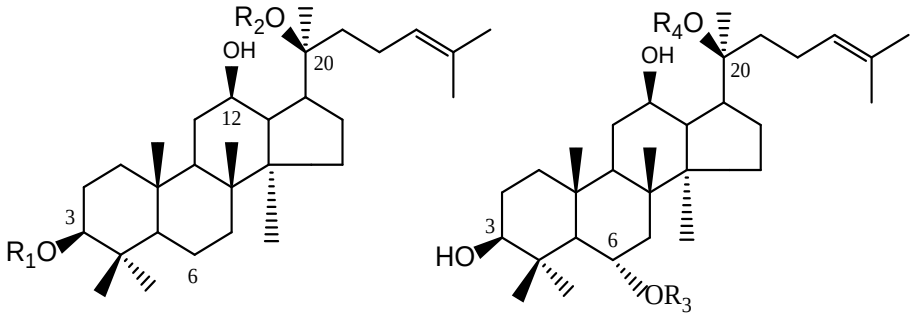
Contemporary science suggests that ginseng has various beneficial effects on diabetes mellitus [3], hyperlipemia [4], hypertension [5], obesity [6], cancer [7], immune disorders [8, 9], cognition impairment [10] and some other diseases [11]. However, there is only preliminary evidence on the beneficial health effects in the human clinical studies. It is considered that various active compounds including ginsenosides, polysaccharides, polyacetylenes, peptides, and flavonoids in ginseng are responsible for its biological and pharmacological activities. However, for the most part, ginsenosides are believed to play a major role in the bioactivities of ginseng

[12]. Some of the active components contained in ginseng are described below.

1.1.1 Ginsenosides

The systematic research on the biological activities of ginseng dates from 1957, when Brekhmam emphasized that the saponin glycoside was the active principle of ginseng [13]. Ever since then, research focused on ginseng saponin began to develop vigorously. From the early 1960s to the middle 1980s, the group of Shibata and Tanaka successively reported that ginseng saponin belonged to triterpenoid compound, with glucose, arabinose, rhamnose or xylose linked to the dammarane structure [14-16]. They named these compounds “ginsenoside Rx” according to their order of polarity on the plate of TLC. Presently it is known that ginsenoside consists of an aglycone and a glycosyl group. Based on the carbon skeleton of the aglycone, ginsenosides can be divided into two groups: the oleanane family and the four-ring dammarane family, which contains the majority of known ginsenosides. The dammaranes can be further subdivided into two types: the PPD-type and the PPT-type, according to the number of hydroxyl groups, which can be joined to sugar moieties by dehydration reaction [17]. The PPD-type has hydroxyl groups at the carbon-3 and carbon-20 positions while the PPT-type at the carbon-3, carbon-6 and carbon-20 positions (Fig. 1-1). The common ginsenosides studied in this work are shown in Table 1-1.

Most studies on the biological effects of ginsenosides have been assessed in cell culture or animal models and thus their relevance to human biology is unknown. Effects on the central nervous system, cardiovascular system, and immune system have been reported, primarily in rodents.

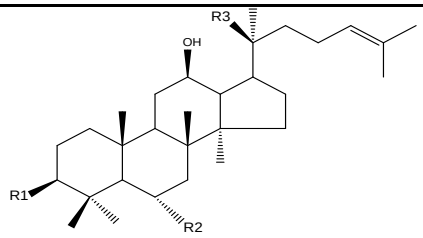


PPD-type PPT-type

Fig. 1-1 Structures of the PPD-type and the PPT-type ginsenosides.

R_1, R_2, R_3, R_4: glycosyl groups.

Table 1-1 Structures of ginsenosides.



	R1	R2	R3
20(S)-Protopanaxadiol-type			
Rb1	O-Glc(2-1)Glc[a]	H	O-Glc(6-1)Glc
Rb2	O-Glc(2-1)Glc	H	O-Glc(6-1)Arap[b]
Rc	O-Glc(2-1)Glc	H	O-Glc(6-1)Arap
Rd	O-Glc(2-1)Glc	H	O-Glc
Rg3	O-Glc	H	OH
F2	O-Glc	H	O-Glc
cK	OH	H	O-Glc
Rh2	O-Glc	H	OH
20(S)-Protopanaxatriol-type			
Re	OH	O-Glc(2-1)Rha[c]	O-Glc
Rg1	OH	O-Glc	O-Glc
Rg2	OH	O-Glc(2-1)Rha	OH
F1	OH	O-Glc	OH
Rh1	OH	OH	O-Glc

[a] β-D-glucopyranosyl; [b] α-L-arabinopyranosyl; [c] α-L-rhamnopyranosyl.

1.1.1.1 Effects of ginsenosides in the central nervous system

Ginsenoside Rb1 facilitates the expression of choline acetyltransferase in the diagonal band of Broca and medial septum of rats [18] and ginsenoside Re attenuates β-amyloid and serum-free induced neurotoxicity in PC12 cells [19]. Chen *et al*. [20] reported that ginsenoside Re, Rg1 and Rg3 effectively inhibited β-amyloid deposition in the brain of mouse. These researches suggest that some ginsenosides might possess beneficial effects on Alzheimer disease.

It was reported that ginsenoside Rb1 and Rg1 alleviated the damage of dopaminergic neuron induced by MPP^+ and significantly increased the numbers and lengths of neurites of surviving dopaminergic cells stressed with glutamate *in vitro* [21, 22]. In fact, some ginsenosides, like Rg1, Rd and Re, have been shown to offer neuroprotection in the model of Parkinson's disease [23, 24].

Some ginsenosides also exert anti-stress effects [25]. Kim *et al*. [26] reported that intraperitoneal administration of ginsenosides Rb2, Rg1, and Rd, as well as the total saponins isolated from ginseng attenuated the immobilization stress-induced increase in plasma IL-6 level. Rai *et al*. [27] showed that ginsenoside Rg3 and Rc might inhibit stress-induced hypothalamo-pituitary-adrenal response by inducing nitric oxide (NO) production in the brain.

In addition, some ginsenosides might exhibit beneficial effects on ischemic encephalopathy. Park *et al*. [28] showed that ginsenoside Rh2 reduced ischemic brain injury in rats. Fujita *et al*. [29] reported that ginsenoside Rb1 protected against damage to the spiral ganglion cells after cochlear ischemia.

1.1.1.2 Effects of ginsenosides in cardiovascular system

Ginsenosides are suggested to dilate blood vessels and thereby lower blood pressure, improve circulation of blood and cerebral blood flow, protect against myocardial infarction, myocardial ischemia and angina, prevent thrombus through inhibiting platelet aggregation, and attenuate chronic renal failure via alleviating arrhythmia and renal ischemia.

Lee *et al*. [30] indicated that the total saponin from Korean red ginseng is a beneficial medicine in platelet-mediated thrombotic diseases via suppressing cyclooxygenase (COX)-1 and thromboxane A2 synthase to inhibit the production of thromboxane A2. The NO has anti-atherosclerotic properties, including inhibition of platelet aggregation, leukocyte adhesion, smooth muscle cell proliferation, and expression of genes involved in atherosclerosis [31]. It was suggested that ginsenosides could stimulate the production of NO by aortic vascular endothelial cells [32]. Ginsenoside Rg3 was shown to cause a concentration-dependent relaxation of rat aortic rings, which might be attributed to an inhibition of Ca^{2+} influx and stimulation of

K^+ efflux. Kim *et al*. [33] reported that intravenous administration of ginsenosides lowered blood pressure in a dose-dependent manner in anesthetized rats. The PPT-type ginsenosides were reported to reduce the blood pressure of hypertensive mice via increasing the release of NO and repressing vasoconstriction factors like superoxide anion and prostaglandin endoperoxide [34].

Ginsenosides were also suggested to improve cerebral blood flow. Tian *et al*. [35] showed that ginsenoside Rg3 might provide neuroprotection against the cerebral ischemia-induced injury in rat brain through reducing the level of lipid peroxides, scavenging free radicals and improving the energy metabolism. Bae *et al*. [36] indicated that cK, ginsenoside Rg3 and Rh2 might improve ischemic brain injury. Kim *et al*. [37] reported that crude saponin from Korean red ginseng enhanced cerebral blood flow in rats.

1.1.1.3 Effects of ginsenosides in immune system

Effects of ginsenosides on humoral immunity, cellular immunity, macrophage activity and release of mediators related to immunoregulation have been reported.

Humoral immunity is mediated by macromolecules found in extracellular fluids such as secreted antibodies, complement proteins, and certain antimicrobial peptides. The humoral immune response to sheep red

blood cells and the phagocytotic function of intraperitoneal macrophages were suppressed by cold water swim stress. Ginseng root saponins, as well as ginsenoside Rb1, completely antagonized the immunosuppression induced by the cold water stress [38]. Song *et al*. [39] reported that ginsenoside Re significantly enhanced serum specific IgG, IgG1, IgG2a and IgG2b responses, lymphocyte proliferation responses as well as IFN-γ and IL-5 secretions in mice challenged by inactivated H3N2 influenza virus antigen equivalent. Qu *et al*. [39] reported that subcutaneous administration of ginsenoside Rg1 developed a high level of specific antibody response against *T. gondii* recombinant surface antigen, a strong lymphoproliferative response, and significant levels of cytokine production.

Cellular immunity is related to the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to antigen. Tong *et al*. [40] showed that ginsenoside Rg1 could promote mitosis in cultured human lymphocytes activated by phytohaemagglutinin (PHA) or Con A. The aging is a declining process associated with dysfunction of neuro-endocrino-immuno-system and the atrophy of thymus plays a critical role in aging with decreased lymphocyte function [41]. Liu *et al*. [42] reported that ginsenoside Rg1 had stimulative effects on the phenotype of lymphocyte and increased the fluidity of the lymphocyte membrane of the aged. Rivera *et al*. [43] demonstrated that ginsenoside Rb1 induced production of large amounts of cytokines

including interferon (INF)-γ, interleukin (IL)-2, IL-4, IL-10 and tumor necrosis factor (TNF)-α and antibodies, and elicited a balanced Th1 and Th2 immune response in mice stimulated by porcine parvovirus vaccines. Natural killer (NK) cells are a type of cytotoxic lymphocyte whose role is analogous to that of cytotoxic T cells. Choi *et al*. [44] suggested that the total ginsenoside from red ginseng might reduce tumor metastasis partially by increasing the activity of NK cells.

Macrophages engulf and digest cellular debris, foreign substances, microbes, cancer cells, and various diseased cells which do not have the types of proteins specific to healthy body cells on their surfaces. The tumoricidal effect of macrophage on K562 tumor cell was increased more by co-treatment with lipopolysaccharide (LPS) and ginseng saponin (44%) than LPS only (22%), and the PPD-type saponin increased the tumoricidal effect by 25-35% and the PPT-type saponin increased it by 55-70% [45]. Ginsenoside Rg1 enhanced the tumor cell killing by NO produced from IFN-γ-activated macrophages [46]. In addition, some ginsenoside might have anti-inflammatory effects in macrophage. Park *et al*. [47] reported that cK, a metabolite of the PPD-type ginsenosides, potently inhibited the production of NO and prostaglandin E2 and suppressed the expression levels of the inducible nitric oxide synthase (iNOS), COX-2 as well as the activation of NF-κB in LPS-activated RAW264.7 cells. Rh1, a metabolite of the PPT-type ginsenoside, was also shown to inhibit iNOS and COX-2

protein expression in RAW 264.7 cells [48].

1.1.1.4 Mechanisms of ginsenosides

Two plausible mechanisms of action on the functional activities of ginsenoside have been suggested based on their similarity to steroid hormones. First, they are amphiphilic and may intercalate into the plasma membrane, which leads to changes in membrane fluidity and thus affects membrane function, thereby eliciting a cellular response [12]. Second, some ginsenosides have been shown to be partial agonists of steroid hormone receptors. It was reported that ginsenoside Rh2 and cK activated glucocorticoid receptor [49, 50], and that Rb1, Rg3 and Rh1 activated estrogen receptor [51-53]. Ginsenoside Rg1 was reported to activate both glucocorticoid receptor and estrogen receptor [54, 55]. However, it is not known how these mechanisms lead to the reported biological effects of ginsenosides.

1.1.2 Polysaccharide

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages. The ginseng polysaccharide, also called ginsan, was reported to show various bioactivities.

Lee *et al*. [56] showed that ginsan induced the proliferation of T cells and B cells, that spleen cells became cytotoxic to a wide range of tumor

cells without major histocompatibility complex (MHC)-restriction after 4 or 5 days culture *in vitro* with ginsan, and that ginsan also activated macrophages to produce reactive nitrogen intermediates and became tumoricidal. Shin *et al*. [57] also reported that when macrophages were treated with ginsan, cytotoxic activity against B16 melanoma cells was significantly induced and the levels of cytokines, including TNF-α, IL-1β, IL-6 and IFN-γ were increased, and that the production of reactive oxygen/nitrogen components such as NO and hydrogen peroxide was enhanced. Ginsan may potentially be an ideal nontoxic antineoplastic immunostimulator by activating multiple effector arms of the immune system. In China, the polysaccharide fraction of ginseng is used clinically in cancer treatment.

In addition, ginsan was also reported to possess hypoglycemic effects. Yang *et al*. [58] showed that ginsan reduced the level of blood glucose and liver glycogen in mice. Xie *et al*. [59] showed that the polysaccharide fraction of American ginseng berry reduced blood glucose level and attenuated glucose intolerance in ob/ob mice.

Moreover, ginsan was shown to have anti-ulcer effect. Sun *et al*. [60] reported that the water-soluble crude polysaccharide fraction from ginseng leaves and the alkaline-soluble crude polysaccharide fraction from ginseng roots prevented HCl/ethanol-induced ulcerogenesis in mice potently.

1.1.3 Polyacetylenes

Polyacetylene usually refers to an organic polymer with the repeating unit $(C_2H_2)_n$. It is known that ginseng contains several kinds of polyacetylene compounds such as panaxynol, panaxydol, panaxydiol, and panaxytriol, some of which are used in Japan as commercial medical drugs. Matsunaga *et al*. [61] reported that these polyacetylenes possess cytotoxic effect. Kwon *et al*. [62] reported that polyacetylene analogs isolated from hair roots of ginseng inhibited acylcoenzyme A: cholesterol acyltransferase (ACAT). Lee *et al*. [63] showed that some polyacetylenes from ginseng inhibited diglyceride acyltransferase (DGAT) in rat liver microsomes.

1.1.4 Peptides

The peptides in ginseng were first reported in 1966, when five small peptides were isolated by electrophoresis [64]. However, these oligopeptides were not pure enough for identification of their sequences. In 1981, Anto *et al*. [65] reported that a heat-stable peptide, which possesses anti-lipolysis effect, was isolated from the aqueous extract of ginseng root. In 1998, Ye *et al*. [66] isolated six γ-glutamyl oligopeptides and identified their sequences for the first time. The peptides from ginseng are considered to exhibit several bioactivities. He *et al*. [67] reported that ginseng oligopeptides had radioprotective effects on intestinal barrier function and antioxidant defense via suppression of TNF-α and reduction of free radicals.

Bao *et al*. [68] indicated that ginseng oligopeptides possessed anti-fatigue effects, which might be attributed to the inhibition of oxidative stress and the improvement of mitochondrial function in skeletal muscles. Ginseng oligopeptides were also suggested to improve the sexual function of male mice by increasing the weight of accessory sex organs, serum levels of testosterone and NO [69].

1.1.5 Flavonoids

Flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and a heterocyclic ring (C), and can be abbreviated C6-C3-C6. Ginseng flavonoids are mainly distributed in the stems and leaves of ginseng. Ginseng flavonoids were identified as kaempferol, trifolin and panaxasenoide [70]. Ginseng flavonoids were reported to influence cardiac performance and hemodynamics. The blood pressure is decreased in the blood vessels in dogs intravenously administered with ginseng flavonoids [71]. Kim *et al*. [72] reported that fermented red ginseng extract containing flavonoid were capable of directly scavenging free radicals, and had anti-oxidative effects in streptozotocin-induced diabetic mice.

1.2 Ginseng and obesity

Obesity is a medical condition in which excess body fat accumulates to the extent that it may have a negative effect on health. Previous researchers have reported that obesity could increase the risk of various diseases, particularly cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, osteoarthritis, asthma and certain types of cancer [73-75]. Many factors such as diet, lifestyle, genetics, and gut microbiota may be associated with obesity; of those, excess food intake is considered as a primary factor [76]. Apart from dieting and physical exercise, several drugs such as lorcaserin, orlistat, phentermine, and topiramate are available for the treatment of obesity. Unfortunately, drug treatment for the amelioration of obesity is often associated with side effects and a rebound weight gain after the cessation of drug use [77]. Complementary and alternative therapies, long used in the Eastern world, are currently receiving considerable attention and are eliciting widespread interest worldwide. Ginseng is an ancient herbal remedy, which was used for approximately 2000 years. Contemporary science suggests that ginseng has various bioactivities. Plenty of studies have indicated that ginseng and ginsenosides might exert a potential anti-obesity effect.

1.2.1 Effect on food intake

Hypothalamic inflammatory activation as a result of consuming a high-

fat diet (HFD) in animal experiments are thought to disturb anorexigenic and thermogenic signals and promote abnormal body weight control [78]. Under chronic inflammation in the hypothalamus of mice, as a response to HFD, a sustained cycle of appetite enhancement were observed [79]. Leptin is a hormone secreted by adipocytes, and it acts on receptors in the arcuate nucleus of hypothalamus to regulate appetite in order to achieve energy homeostasis. Long-term HFD consumption in rodents has been reported to evoke leptin resistance, which is characterized by an increased level of plasma leptin. Ginsenoside Rb1 was reported to decrease the expression levels of inflammatory markers such as p-IκB kinase, IL-6 and IL-1β and negative regulators of leptin signaling such as suppressor of cytokine signaling (SOCS) 3 and protein-tyrosine phosphatase 1B (PTP1B) in hypothalamus and restore the anorexic effect of leptin and leptin p-STAT3 signaling in hypothalamus of HFD-fed mice [80]. Administration of ginseng extracts has decreased plasma levels of leptin and neuropeptide Y (NPY) and alleviated leptin resistance in HFD-fed murine [53]. In addition, it was reported that PPD-type ginsenosides inhibited the expression of cholecystokinin, which acts as a hunger suppressant, in hypothalamus of mice fed a HFD while PPT-type ginsenosides increased the expression [81]. Through such actions, ginseng or ginsenosides may prevent excess energy intake and the onset of obesity. In support of that suggestion, a number of animal researches have indicated that ginseng administration could repress

food intake in rodents [6, 81-88].

1.2.2 Effect on digestion and absorption systems

Liu *et al*. [89] reported that PPD-type ginsenosides such as Rb1, Rb2, Rc, and Rd significantly suppressed pancreatic lipase activity while PPT-type ginsenosides Re and Rg1 do not, which supported the research results reported by Liu *et al.* [90]. In addition, an extract of ginseng root, mainly containing the PPD-type ginsenosides [91], was shown to exert similar activities [89, 92]. Pancreatic lipase inhibitors can prevent obesity by increasing fat excretion into feces, and it has been reported that supplementation of ginseng extract increases fecal weight and fecal lipid content in mice [83, 93]. Therefore, ginseng may decrease energy harvest of an organism by inhibiting pancreatic lipase activity. Although the PPD-type ginsenosides may be more effective than the PPT-type considering the inhibitory effect on pancreatic lipase activity, the PPT-type ginsenoside Rg1 was shown to suppress the expression of sodium-glucose linked transporter (SGLT1), thereby decreasing glucose absorption across Caco-2 cell monolayer, whereas cK, a PPD-type ginsenoside, increased the expression of SGLT1 and the uptake of glucose [94]. Subsequent research has revealed that ginsenoside Rg1 can inhibit SGLT1 expression by reducing the binding of cAMP response element-binding protein (CREB) to the cAMP response element, which is associated with an inactive chromatin status [95].

1.2.3 Effect in liver

The enzyme AMP-activated protein kinase (AMPK) acts as a metabolic master switch regulating cellular energy homeostasis. The activation of AMPK stimulates fatty acid oxidation, ketogenesis as well as biogenesis of mitochondria and uptake of glucose, but inhibits cholesterogenesis, lipogenesis, and triglyceride synthesis [96].

Numerous *in vitro* researches have documented that ginseng and ginsenosides could activate the AMPK pathway, resulting in increased levels of p-AMPK and p-ACC in hepatocyte HepG2 cells [97-105] (Table 1-2). By activating this pathway, ginseng and ginsenosides can, *in vitro*, suppress the expression of fatty acid synthase (FAS), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), phosphoenolpyruvate carboxykinase (PEPCK), and glucose 6-phosphatase (G6Pase), thereby inhibiting triglyceride synthesis [97, 98, 101], cholesterogenesis [98, 103] and gluconeogenesis [99, 100, 104].

Consistent with the results of *in vitro* studies, various *in vivo* animal studies have indicated that ginseng or ginsenosides activate the AMPK pathway in liver in a HFD-fed animal model [99, 106]. The HFD-fed mice supplemented with ginseng extract showed a low liver weight [107, 108], which might be attributed to a decrease in the deposition of hepatic lipid. In support of that suggestion, several researchers have reported that ginseng

supplementation can decrease hepatic lipid content and ameliorate hepatic steatosis [82, 83, 87, 88, 93, 107-109] (Table 1-3).

Peroxisome proliferator-activated receptor (PPAR)-α can be activated downstream by AMPK and can facilitate fatty acid export from hepatocytes and oxidation [110]. It has been reported that fermented ginseng extract increased the expression of PPAR-α in HepG2 cells [97]. Furthermore, ginseng extract as well as its main ginsenoside Rb1 exerts such a similar effect *in vivo* [88, 111]. HFDs increase PPAR-γ protein expression and decrease the expression of CREB in the nuclei of hepatocytes, which is associated with HFD-induced liver steatosis [112]. Ginsenoside PPT, the final metabolite of PPT-type ginsenosides, acts as a PPAR-γ antagonist and represses fat deposition in the liver of HFD-induced obese mice[84].

Non-alcoholic fatty liver disease (NAFLD), the most common liver disorder in developed countries, occurs when fat is deposited in the liver due to causes other than excessive alcohol use. Up to 80% of evaluated obese individuals have been shown to have NAFLD [113]. The NAFLD is strongly associated with hepatic insulin resistance and type 2 diabetes [114]. On a HFD, lipotoxicity can result in increased activities of aspartate transaminase (AST) and alanine aminotransferase (ALT), which are commonly measured as clinical biomarkers to evaluate liver function. The HFD-fed mice supplemented with ginseng showed low activity levels of these two enzymes [108]. Ginseng might, therefore, alleviate lipotoxicity,

hepatic steatosis, and insulin resistance by activating the AMPK pathway.

In enterohepatic circulation, bile synthesized in liver from cholesterol is released to the intestine, where a portion of the bile acids is degraded by intestinal bacteria exerting bile acid hydrolase activity and excreted with feces [115]. Cholesterol is used to neo-synthesize bile acids in a homeostatic response, resulting in a lower level of cholesterol in liver and plasma. Cholesterol 7 alpha-hydroxylase (CYP7A1) and sterol 12 alpha-hydroxylase 1 (CYP8B1) are enzymes involved in bile acid synthesis, and multidrug resistance-associated protein 2 (MRP2) is a transporter that facilitates biliary efflux from hepatocytes. It has been shown that red ginseng extract as well as ginsenosides can increase the expression of CYP7A1, CYP8B1 and MRP2 both *in vitro* and *in vivo* [116, 117]. Ginsenoside Rb1 can decrease the cholesterol content in liver of HFD-fed mice by suppressing HMGCR [118], and ginsenoside Rb2 can up-regulate the expression of low density lipoprotein receptor (LDL-R), which mediates the clearance of cholesterol from plasma to hepatocytes [119, 120]. Qureshi *et al.* [109] and Marwan *et al.* [121] showed that dietary supplementation of ginseng can suppress avian hepatic cholesterogenesis and decrease plasma LDL cholesterol. Taken together, it may be concluded that ginseng inhibits cholesterogenesis in liver and facilitates cholesterol clearance in plasma, bile acid synthesis from cholesterol, and biliary efflux from hepatocytes. Through the combination of these effects, the levels of

cholesterol in liver and plasma might be regulated.

Table 1-2 Effects of ginseng on different targets related to obesity in cell line studies.

Material	Cell line	Mechanism	Ref.
Rb1		insulin-induced GPDH↑	
Rb1, Rd, Rh2 Re, Rg1, Ro	3T3-L1	insulin-induced adipogenesis↑ no effect	[122]
Rb2,Ro, Re, Rg1, Rh1	3T3-L1	LPL↑	[123]
Rb1	3T3-L1	PPAR-γ↑, C/EBPα↑, aP2↑,GLUT4↑ adipogenesis↑	[124]
PPT	3T3-L1	PPAR-γ↑, aP2↑, LPL↑, GLUT4↑, PEPCK↑, adipogenesis↑	[125]
PPT	3T3-L1 (rosiglitazone)	PPAR-γ↓, aP2↓, C/EBPα↓, FAS↓, CD36↓, LPL↓	[84]
Rh2	3T3-L1	PPAR-γ↓,p-AMPK↑, ROS↑,UCP2↑, CPT1↑, adipogenesis↓	[126]
Rb2, Rc, Rd, Re, Rb1, Rg1	3T3-L1	TAG↓, cAMP↓ glucose uptake↑ cAMP↑, PKA↑, PPAR-γ↓, C/EBPα↓, aP2↓, TAG↓, glucose uptake↑	[127]
Rb1	3T3-L1	GLUT1 and GLUT4 translocation↑, IRS1↑, p-Akt↑, PI3K↑ Glucose uptake↑	[128]
Rg3	3T3-L1	PPAR-γ↓, AMPK↑, adipogenesis↓(rosiglitazone-treated)	[129]
Rb2	3T3-L1	in high cholesterol and high fatty acids conditions, SREBP1↑, FAS↑, leptin↑, cholesterol ↓, TAG↓	[130]
Rg1 cK	3T3-L1	GLUT4↑, p-Akt↑,p-AMPK↑, p-ACC↑, glucose uptake↑, TAG↑ GLUT4↑, p-Akt↑,p-AMPK↑, p-ACC↑, glucose uptake↑, TAG↓	[131]
Rh2	3T3-L1	activation of glucocorticoid receptor↑, adipogenesis↑	[50]
Rg3, less polar ginsenosides	3T3-L1	Lipid accumulation↓	[132]
Re	3T3-L1	TNF-α↓, LPL↑, leptin↓, resistin↓	[133]
Re, Rc	3T3-L1	leptin↓, HSL↑, resistin↓,	[134]
American ginseng	3T3-L1	adiponectin↑, TAG↓	[135]
Ginseng extract	3T3-L1	adiponectin↑, TAG↓	[136]
Re, Rg3	3T3-L1	GLUT4↑, IRS1↑,PI3K↑, glucose uptake↑	[137]
cK	3T3-L1	PPAR-γ↓, aP2↓, C/EBPα↓,VEGF-A↓, FGF2↓,MMP2↓, MMP9↓, TSP1↑, TIMP1↑, TIMP2↑, adipogenesis↓	[138]
Ginseng extract, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3	3T3-L1	PPAR-γ↓, aP2↓, C/EBPα↓, MMP2↓, MMP9↓, TIMP1↑, TIMP2↑, adipogenesis↓	[139]
Rb2	HepG2	SREBP1↑, LDL-R↑	[119]
Rg1	HepG2	p-AMPK↑, p-ACC↑, G6Pase↓, PEPCK↓, gluconeogenesis↓	[100]
Fermented ginseng	HepG2	PPAR- α ↑, p-AMPK↑, p-ACC↑, FAS↓,TAG↓	[97]
Korean red ginseng	HepG2	p-AMPK↑, p-ACC↑, FAS↓, SCD↓, TAG↓	[101]
Korean red ginseng	HepG2 L6 myotubule	p-AMPK↑, p-ACC↑ p-AMPK↑, p-ACC↑	[102]
Rg3	HepG2	SREBP2↓, HMGCR↓, cholesterol↓, TAG↓, AMPK↑	[103]
Re	HepG2	p-AMPK↑, p-ACC↑, G6Pase↓, PEPCK↓, SREBP1↓, FAS↓, gluconeogenesis↓	[99]
Rg1	HepG2	p-Akt↑, p-AMPK↑, p-ACC↑, gluconeogenesis↓, glycogen synthesis↓, lipids↓	[104]
Korean red ginseng	HepG2	FAS↓, HMGCR↓, TAG↓, cholesterol↓	[98]
ginseng	HepG2	p-AMPK↑, FAS↓, HMGCR↓, TAG↓, TC↓	[105]
Rc	C2C12	p-AMPK↑, p-ACC↑, glucose uptake↑	[140]
Rg1	C2C12	AMPK↑, p-AMPK↑, GLUT4↑, glucose uptake↑	[141]
Korean red ginseng	C2C12	p-AMPK↑, p-ACC↑, fatty acid oxidation↑	[142]
ginseng extracts	C2C12	Glucose uptake↑	[143]
Re, Rc	C2C12	p-AMPK↑, glucose uptake↑	[144]
20(R)Rg3	C2C12	p-AMPK↑, p-ACC↑, glucose uptake↑	[145]
Rg3, Rh2	C2C12	AMPK↑, glucose uptake↑	[146]
Rb1	C2C12	AdipoR1↑, AdipoR2↑, GLUT4↑,	[147]
Rg3	C2C12	IRS1↑, p-Akt↑, ATP↑, PGC1- α↑, NRF1↑	[148]
black ginseng	C2C12	p-IRS1↑, p-LKB1↑, p-AMPK↑, p-mTOR↑	[85]

aP2, adipocyte protein 2; CPT, carnitine palmitoyltransferase; GPDH, glycerol-3-phosphate dehydrogenase; HSL, hormone sensitive lipase; LKB1, liver kinase B1; mTOR, mechanistic target of rapamycin; PI3K, phosphatidylinositide 3-kinases; PKA, protein kinase A; ROS, reactive oxygen species; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein; UCP, uncoupling protein.

1.2.4 Effect in adipose tissue

There are several reports showing that ginseng can reduce adipocyte size and fat storage in mice and rats fed a HFD [53, 90, 149, 150]. In fact, ginseng or ginsenosides also activate the AMPK pathway in fat cells. Ginsenoside Rg1, Rg3, Rh2, and cK increase the level of p-AMPK and inhibit triglyceride synthesis in 3T3-L1 cells [126, 129, 131]. PPAR-γ stimulates lipid uptake, fatty acid storage, and adipogenesis in fat cells, and PPAR-γ knockout mice fail to generate adipose tissue when fed a HFD [151]. It has also been reported that ginsenosides Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, and cK suppressed PPAR-γ and C/EBP-α, thereby inhibiting adipogenesis in 3T3-L1 cells [129, 130, 132, 138, 139]. With regard to the effects of ginsenosides Rb1, Rd, Rh1, and PPT on adipogenesis *in vitro*, the results of previous studies have been inconsistent [50, 84, 122, 124-127, 139], which might be attributed to the distinct experimental conditions. The HFD model studies have indicated that ginseng repressed differentiation of fat cells in adipose tissue of rodents and produced an anti-obesity effect [53, 93, 152], whereas *ob*/*ob* and *db*/*db* diabetic mouse studies have shown that ginseng treatment stimulated the expression of PPAR-γ, adipogenesis, and exerted insulin-like effects [111, 153]. Lipoprotein lipase (LPL) releases free fatty acids from circulating triglyceride-rich lipoprotein and mediates the clearance of blood fats. Ginsenosides Ro, Rb2, Re, Rg1, and Rh1 were suggested to increase insulin-induced expression of LPL [123], whereas the

results from PPT-type ginsenosides were contradictory [84, 125]. Ginseng treatment down-regulates the expression of LPL in HFD fed mice [53], but up-regulates it in diabetic ob/ob or db/db mice [111, 153]. Ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1, Rg3, and cK have been shown to stimulate glucose uptake in 3T3-L1 cells [127, 128, 131, 137]. Taken together, these results suggest that ginseng and ginsenosides may have biphasic modulation effects on PPAR-γ, LPL, and adipogenesis and may have a modulatory effect on the maintenance of homeostasis.

Adiponectin, exclusively secreted from adipose tissue, is a protein hormone that modulates fatty acid oxidation and glucose regulation and adiponectin levels are inversely correlated with body fat percentage in adults. Ginseng was shown to significantly increase the secretion of adiponectin in 3T3-L1 cells and in mice fed a HFD [84, 107, 135, 136, 154]. Resistin is an adipose-derived hormone, and its function has been the subject of controversy with respect to its involvement in obesity. Serum resistin levels increase with increased adiposity and decline with decreased adiposity [155], and it has been shown that the ginsenosides Rc and Re can repress resistin expression in 3T3-L1 cells [133, 134].

Unlike other tissues, which stop growing in adulthood, adipose tissue can grow and regress throughout life. Adipose tissue is highly vascularized and adipocytes are nourished by an extensive capillary network, which suggests that obesity might be blocked by angiogenesis inhibitors. Matrix

metalloproteinases (MMPs) are thought to play a major role in adipogenesis and angiogenesis. *In vitro* studies have demonstrated that ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, and cK suppress the expression of vascular endothelial growth factor A (VEGF-A), basic fibroblast growth factor 2 (FGF2), and MMPs, whereas they facilitate the expression of angiogenic inhibitors such as thrombospondin 1, tissue inhibitor of metalloproteinase (TIMP) 1 and TIMP2 in 3T3-L1 cells [138, 139]. Such effects of ginseng on adipose tissue differentiation were also observed in HFD-induced obese mouse studies [6, 87].

Obesity is associated with hyperplasia and hypertrophy of adipose tissue and is likely to lead to a reduction of adipose tissue blood flow, which results in adipocyte hypoxia [156]. Adipose hypertrophy, the enlargement of adipocytes, can increase the distance from adipocytes to blood capillaries, resulting in adipocyte hypoxia. Increased necrosis-like adipocyte cell death due to hypoxia has been reported to result in recruitment of macrophages to adipose tissue [157]. Macrophages surrounding dying or dead adipocytes form crown-like structures that can be identified by the absence of perilipin staining. Activated adipocytes and macrophages release proinflammatory cytokines such as IL-6 and TNF-α, and they promote insulin resistance [158]. Kim *et al*. reported that the ginsenoside Re can repress the expression of TNF-α in 3T3-L1 cells [133]. Moreover, ginsenoside Rh1 was shown to prevent macrophage infiltration and decrease the release of IL-6, TNF-α,

and IL-1β in the adipose tissue of HFD-induced obese mice [152]. Extracts of ginseng have also been found to repress the secretion of TNF-α, IL-6, and monocyte chemoattractant protein 1 (MCP1) in the adipose tissue of mice fed a HFD [93].

Table 1-3 Effects of ginseng on different targets related to obesity in animal studies.

Material		Animal	Mechanism	Ref.
Ginseng extracts	orally, 4 week	chickens	BW gain↓, serum TC↓, LDL-C↓, TAG↓, liver HMGCR↓, CYP7A1↓, FAS↓	[109]
Korean red ginseng	i.p. 3 weeks	HFD rats	food intake↓, BW gain↓, fat storage↓, leptin↓, NPY↓	[149]
Wild ginseng extract	8 weeks	HFD mice	BW gain↓, serum FBG↓, IR↓, TAG↓, TC↓, HDL-C↑, LDL-C↓, NEFA↓, adipocyte size↓, adipose tissue GLUT4↓	[150]
Mix of PPD type ginsenosides	Orally, 8 weeks	HFD mice	BW gain↓, liver weight↓, adipose tissue weight↓, serum TAG↓, TC↓, LDL-C↓, liver TAG↓, TC↓	[90]
Ginseng extract	Orally, 8 weeks	HFD mice	BW gain↓, weight of WAT↓, serum TAG↓, leptin↓, adipocyte size↓, PPAR-γ↓, SREBP1↓, FAS↓, LPL↓, DGAT1↓	[53]
Vinegar processed Ginseng extracts	Orally, 8 weeks	HFD mice	food intake↓, BW gain↓, FBG↓, insulin↓, HOMA-IR↓, liver weight↓, fat weight↓, serum TAG↓, TC↓, LDL-C↓, NEFA↓, HDL-C↑, blood pressure↓, adipocyte size↓	[82]
Ginseng saponin	Orally, 3 weeks	HFD mice	BW gain↓, serum TAG↓	[92]
PPD type PPT type	i.p. 3 weeks	HFD rats	food intake↓, BW gain↓, fat storage↓, serum TAG↓, TC↓, HDL-C↑, leptin↓, NPY↓, CCK↑(PPD), CCK↓(PPT)	[81]
Korean red ginseng	Orally, 13 weeks	HFD mice	BW gain↓, liver weight↓, fat storage↓, serum TC↓, LDL-C↓, leptin↓, insulin↓, adiponectin↑	[107]
Korean red ginseng	Orally, 8 weeks	HFD mice	food intake↓, BW gain↓, fat storage↓, adipocyte size↓, blood vessel density↓, MMP2↓, MMP9↓, VEGF-A↓, FGF2↓, TSP1↑, TIMP1↑, TIMP2↑	[6]
Ginseng radix	Orally, 8 weeks	HFD mice	BW gain↓, FBG↓, insulin↓, HOMA-IR↓, muscle p-AMPK↑, p-ACC↑, GLUT4↑,	[106]
Ginsenoside Re	Orally, 3 weeks	HFD mice	FBG↓, insulin↓, HOMA-IR↓, NEFA↓, Liver p-AMPK↑, p-ACC↑, SREBP1↓, FAS↓, GPAT↓, PEPCK↓, G6Pase↓.	[99]
Korean red ginseng	Orally, 12 weeks	HFD rats	BW gain↓, fat storage↓, adiponectin↑, leptin↓, muscle p-IRS1↑, p-Akt↑, p-GSK↑, GLUT4↑	[154]
Black ginseng	Orally, 12 weeks	HFD mice	food intake↓, BW gain↓, fat storage↓, fecal weight↑, fecal lipid↑, liver lipid↓	[83]
Fermented Korean red ginseng	Orally, 12 weeks	HFD mice	BW gain↓, adipocyte size↓, serum TC↓, TAG↓, LDL-C↓, hepatocyte size↓, liver steatosis↓, AST, ALT↓	[108]
Ginsenoside Rh1	Orally, 4 weeks	HFD mice	BW gain↓, adipocyte size↓, PPAR-γ↓, aP2↓, C/EBPα↓, FAS↓, TNF-α↓, IL-1β↓, IL-6↓, CD68↓, F4/80↓,	[152]
Ginseng extract	Orally, 14 weeks	HFD rats	BW gain↓, epididymal fat↓, serum TAG↓, leptin↓, liver TAG↓, fecal TAG↑, adipose tissue PPAR-γ↓, aP2↓, TNF-α↓, IL-6↓, MCP-1↓	[93]
Ginseng radix	Orally, 5 weeks	HFD mice	food intake↓, BW gain↓, epididymal fat↓, adipocyte size↓, FBG↓, insulin↓, HOMA-IR↓, serum TAG↓, TC↓, NEFA↓, muscle p-AMPK↑, p-ACC↑, GLUT4↑	[86]
PPT	Orally, 4 weeks	HFD mice	food intake↓, BW gain↓, FBG↓, serum TAG↓, TC↓, LDL-C↓, NEFA↓, insulin↓, leptin↓, adiponectin↑,IL-1 β ↓, IL-6↓, AST↓, ALT↓, Liver TAG↓ TC↓,FAS↓, body temperature↑, adipose UCP1↑, UCP2↑, UCP3↑, TNF-α↓, IL-6↓, IL-1 β ↓	[84]
Rb1	i.p. 12 weeks	HFD rats	Food intake↓, liver TAG↓, p-AMPK↑, CPT1↑, β-oxidation↑, SREBP1↓, FAS↓, SCD1↓, PGC1 α↑, PPAR-α↑, Acox1↑	[88]
Korean red ginseng	Orally, 12 weeks	db/db mice	BW gain↓, FBG↓, insulin↓, HbA1c↓, serum TAG↓, liver PPAR- α ↑, adipose tissue PPAR-γ↑, LPL↑	[111]
Ginseng berry Ginseng root	i.p. 12 days	ob/ob mice	food intake↓, BW gain↓, FBG↓, body temperature↑; BW gain↓, FBG↓	[159]
Wild ginseng	Orally 4 weeks	ob/ob mice	BW gain↓, FBG↓, adipose tissue PPAR-γ↑, LPL↑, GLUT4↑, Liver GLUT4↑, IR↑, muscle LPL↑, GLUT4↑, IR↑	[153]
ginseng	Orally 13 weeks	db/db mice	food intake↓, BW gain↓, adipocyte size↓, hepatic lipids↓, serum TAG↓, NEFA↓, FBG↓, insulin↓; adipose tissue blood vessel density↓, VEGF-A↓, FGF-2↓, UCP2↑, CPT-1↑	[87]

Acox1, peroxisomal acyl-coenzyme A oxidase 1; BW, body weight; DGAT1, diglyceride acyltransferase; FBG, fasting blood glucose; GPAT, glycerol-3-phosphate O-acyltransferase; GSK, glycogen synthase kinase; HOMA-IR, homeostatic model assessment-insulin resistance; IR, insulin resistance; NEFA, non-esterified fatty acid; TC, total cholesterol; WAT, white adipose tissue.

1.2.5 Effect in skeletal muscle

Skeletal muscle is the predominant tissue responsible for oxidation of glucose and fatty acids and therefore is a potential target for anti-obesity and anti-diabetes therapies. The AMPK is an important energy-sensing and signaling system in skeletal muscle. Once activated, it may stimulate the biogenesis of GLUT4 and mitochondria and facilitate glucose uptake and acute fatty acid oxidation via phosphorylation of ACC with a consequent decrease in malonyl-CoA [160]. Many *in vitro* studies have indicated that ginsenosides Rc, Re, Rg1, Rg3, and Rh2 and ginseng extracts can activate the AMPK signaling pathway in C2C12 myoblast cells [85, 140-142, 144-146]. In addition, it has been reported that ginseng root can activate AMPK in skeletal muscle of mice fed a HFD [86, 106]. In that manner, ginsenosides or ginseng can alleviate insulin resistance via increased phosphorylation of IRS-1 and Akt and facilitate uptake of glucose to myocytes via the regulation of Glucose transporter type 4 (GLUT4) biogenesis [128, 143, 154]. Korean red ginseng was reported to promote mitochondrial biogenesis and fatty acid oxidation in skeletal muscle and cultured C2C12 cells with increased expressions of PPAR-γ coactivator-1α (PGC-1α), nuclear respiratory factor 1 (NRF-1), cytochrome c, and cytochrome c oxidase [142, 148, 154]. Jung *et al*. [161] assessed the rate of glucose transport in the epitorchealis muscle under submaximal insulin concentrations and did not detect incremental glucose uptake after ginseng

treatment. However, the rats in their study were fed a HFD and treated with ginseng for only 2 weeks, thus their research design might be a reason for their contrasting result. The AMPK can be regulated downstream by adiponectin, and ginsenoside Rb1 has been shown to stimulate adiponectin signaling in C2C12 muscle cells through up-regulation of AdipoR1 and AdipoR2 proteins [147].

1.2.6 Human study

Only 7 papers of human study associated with ginseng and obesity are available and reviewed (Table 1-4). Kim *et al*. [162] reported that serum levels of total cholesterol (TC), triglyceride, and LDL decreased while HDL increased following the administration of ginseng extract for 8 weeks. A limitation of their study is that it was not placebo-controlled. Dominic *et al*. [163] reported that oral administration of ginsenoside Re or Korean red ginseng extract to obese adults failed to have an effect on body weight, body mass index (BMI), fat mass, and plasma lipid profile. Although the small number of study subjects (n = 5) may be a limitation of that research, their data did not even detect a trend toward treatment-induced improvement. Kwon *et al*. [164] also reported that the administration of Korean red ginseng extract to obese females at a dose of 6 g/d for 8 weeks failed to show an effect different from that in their placebo group, with the exception of an improvement in the obesity-related quality of life scale. Similarly, Cho *et al*. [165] reported that administration of Korean red ginseng powder to

overweight or obese adults at a dose of 6 g/d for 12 weeks did not have an effect on BMI, body fat, and plasma lipid profile. In addition, Park *et al.* [166] reported that administration of Korean red ginseng to adults with metabolic syndrome at a dose of 4.5 g/d for 12 weeks failed to have an effect on waist circumstance, lipid profile, and insulin resistance. In contrast, Song *et al.* [167] reported that administration of ginseng extract to obese middle aged females at a dose of 8 g/d for 8 weeks did produce a weight loss effect; moreover, there were slight effects on gut microbiota with the anti-obesity effects differing dependent on the composition of the gut microbiota prior to ginseng administration. However, their research design was limited by the absence of a placebo control. In male subjects with metabolic syndrome, Jung *et al.* [168] reported that red ginseng improved mitochondrial function, increased levels of testosterone and insulin-like growth factor 1(IGF-1), and reduced both diastolic blood pressure and serum cortisol level compared to the results in their placebo group.

Notably, ginsenosides have a very low bioavailability after oral intake and only the deglycosylated forms of ginsenosides can be absorbed into the circulatory system. The transformation of ginsenosides is largely dependent on intestinal bacteria, which release various glycosidases to hydrolyze the sugar moieties of ginsenosides. Intestinal microflora composition varies among individuals, and approximately 20% of people cannot partially or fully transform ginsenosides [169]. Moreover, the degree of transformation

and concentration of ginsenosides may vary among ginseng products. In addition, the effects of ginseng might vary with individual genotypes [164]. These factors may, in part, lead to the differing results attained in the various human-based research carried out thus far. In addition, the treatment periods have usually been 8 weeks, regardless of whether the study is animal or human based. As the human life span is far longer than that of murine, such a short treatment period may be another reason for the apparent lack of anti-obesity effects in human studies.

Table 1-4 Effects of ginseng on different targets related to obesity in human studies.

Material	Subject	Mechanism	Ref.
50% alcohol ginseng extract 6 g/d, for 8 weeks	male college student n=8	MDA↓, SOD↑, CAT↑, TC↓, HDL↑, LDL↓, TAG↓	[162]

Korean red ginseng extract
3g/d for 2 weeks, 8g/d for 2 weeks;
Ginsenoside Re,
0.25g/d for 2 weeks, 0.5g/d for 2weeks
	obese adults
	placebo, n=5;
	intervention, n=5;
	Re, n=5
		no effect on weight, BMI, fat mass, glucose, insulin, HbA1c, TC, TAG, HDL, LDL no effect	[163]

Korean red ginseng extract 6 g/d for 8 weeks	obese females placebo n=23; intervention, n=22	BW↓, BMI↓, WHR↓, food intake↓, Genotype: GNB3, CT: BMI↓, WHR↓, food intake↓, SBP↓; ADRB3, Trp64/Arg: BST ↓ Trp64/Trp: AST↓; ACE, II: BST↓, AST↓, no distinct effect compared to placebo	[164]
Korean red ginseng powder 6 g/d for 12 weeks	overweight or obese adults placebo n=34; intervention, n=34	no effect on caloric intake, BMI, percent body fat Blood TC, TAG, LDL, HDL	[165]
Korean red ginseng 4.5 g/d for 12 weeks	adults with metabolic syndrome placebo n=25; intervention, n=23	no effect on waist circumference, blood pressure, TC, HDL, TAG, fasting plasma glucose, insulin, HOMA-IR	[166]
Panax ginseng extract 8 g/d for 8 weeks	obese females n=10	weight gain↓, BMI↓, no effect on waist circumference, body fat percentage, plasma HDL, TAG, TC and glucose. effects differed depending on the composition of gut microbiota prior to ginseng intake	[167]
Red ginseng 3 g/d for 4 weeks	males with metabolic syndrome placebo n=30; intervention, n=32	mitochondrial function↑, total testosterone↑ IGF-1↑, diastolic blood pressure↓	[168]

CAT, catalase; IGF-1, insulin-like growth factor 1; MDA, malondialdehyde; SOD, superoxide dismutase; WHR, waist–hip ratio.

1.2.7 Conclusion

Ginseng or ginsenosides may help control appetite and prevent the over intake of food energy by attenuating the HFD-induced chronic inflammation in the hypothalamus, improving leptin resistance, and reducing the secretion of NPY. Once food is consumed, PPD-type ginsenosides can inhibit the activity of pancreatic lipase and prevent digestion of triglyceride. Ginsenoside Rg1 suppresses the expression of SGLT1 and blocks the absorption of glucose. In this way, the energy harvested by an organism from the consumed lipids and carbohydrates can be reduced. Through the activation of AMPK, metabolism is switched from anabolism to catabolism. In liver, triglyceride synthesis, cholesterogenesis, and gluconeogenesis are down-regulated through the suppression of FAS, HMGCR, PEPCK, and G6Pase. Moreover, PPAR-α is activated downstream by AMPK, and it stimulates oxidation and export of fatty acids. In this way, liver steatosis induced by HFD may be improved. Furthermore, ginseng and ginsenosides stimulate the synthesis of bile acid from cholesterol, up-regulate the expression of LDL receptor, and thereby mediate cholesterol clearance from blood and liver. Ginseng and ginsenosides also activate the AMPK pathway and inhibit triglyceride synthesis in adipose tissue. Results describing the effects of ginseng on adipogenesis via PPAR-γ and C/EBPα have so far been inconsistent. However, many researchers have reported that HFD-fed mice administrated

with ginseng have lower adipose tissue weights and small adipocytes. Ginseng and ginsenosides may have a dual regulatory effect on adipogenesis and maintain homeostasis of lipid metabolism. In addition, inflammation due to hypoxia in adipose tissue is ameliorated by ginseng. Ginseng and ginsenosides also stimulate the AMPK pathway in skeletal muscle. Glucose uptake and fatty acid oxidation are up-regulated via stimulation of GLUT4 and mitochondria biogenesis. Ginseng may down-regulate blood glucose and lipids by facilitating energy expenditure in muscle.

In summary, ginseng and ginsenosides not only modulate appetite and reduce energy input in the intestine, but also inhibit lipid synthesis and stimulate energy consumption in skeletal muscle and liver via the activated AMPK pathway. Therefore, to some extent, the anti-obesity effect of ginseng may be explained by the principle of energy conservation. In addition, ginseng treatment can result in a two-way adjustment of adipogenesis under HFD-induced obese and diabetic conditions. Nevertheless, previous studies into the anti-obesity effects of ginseng are mostly restricted to animals. There is limited evidence supporting the notion that ginseng exerts an anti-obesity effect in humans. Additional study and verification through longitudinal human studies are required to elucidate the anti-obesity effects of ginseng in humans.

1.3 ginseng root and ginseng berry

Ginsenosides are distributed in many parts of the ginseng plant, including the root, leaf, and berry. Different parts of the plant contain distinct ginsenoside profiles, and these parts may have different bioactivities. It is known that the ginsenoside content in the berry is much higher than that in the root [170]. It is worth noting that Re, a PPT-type ginsenoside, in the berry amounts to dozens of times that of ginseng root [171]. The most abundant ginsenoside in the berry is Re while the most abundant ginsenoside in the root is Rb1, a PPD-type ginsenoside [91].

As a by-product of ginseng, ginseng berry can be harvested several times during the period of cultivation. Ginseng berry extract contains larger amounts of vitamin E, vitamin K, folic acid, and potassium and currently, ginseng berry extract is being evaluated in preclinical and clinical trials because its components are more efficacious as compared to ginseng root extract [171].

1.4 Transformation of ginsenosides

As previously mentioned, ginsenosides contain an aglycone and several sugar moieties. Remove of the sugar moieties from the aglycone transforms the precursor ginsenoside to a relatively less polar ginsenoside. This process is called transformation of ginsenoside.

The biological activities of ginsenosides are based on the number and position of sugar moieties linked to the aglycone. Ginsenosides with more glycosyl groups may show a low bioavailability due to their high polarity while ginsenosides with less glycosyl groups may penetrate through the hydrophobic phospholipid bilayer of enterocyte more easily.

After ingesting herbal medicine, the main active ingredients are transformed to their nonpolar forms by the intestinal microflora before absorption via the enterocytes in the gastrointestinal tract [172, 173]. Tawab *et al*. [174] reported the ginsenoside cK, Rh1 and F1, rather than the undeglycosylated forms that were detected in human plasma and urine after ginseng powder was administrated to volunteers. Therefore, the biological activities of ginsenosides may largely depend on the metabolic activity of intestinal microflora. Since the intestinal microflora vary among individuals, about 20% of people cannot efficiently, or even at all, transform ginsenosides [169]. This may explain why some people using ginseng achieved their expectations while others did not. The deglycosylation

degree of ginsenosides may also be one of the reasons why commercial ginseng products vary in effects. Moreover, deglycosylated ginsenosides show more efficient activity than the undeglycosylated forms [175]. Consequently, it is necessary to transform ginsenosides before ingesting purely ginseng.

1.4.1 Transformation of ginsenosides by heating

At relatively high temperature (100-120℃), ginsenosides can degrade to yield the deglycosylated forms of ginsenosides. In fact, fresh ginseng is usually used to produce commercial red ginseng by the process of heating. Ginsenoside Rg3, one of the characteristic constituents in red ginseng, is produced from its precursors like ginsenoside Rb1, Rb2, Rc and Rd during the steaming process [176]. Kim *et al.* [177] found that after the raw ginseng was steamed at 120℃ for 2 h using an autoclave, the levels of ginsenoside Rb1, Rb2, Rc, Rd, Re and Rg1 decreased, while the levels of ginsenoside F4, Rg3, and Rg5 increased. Ginsenosides Rg3 and Rg5 were the most abundant ginsenosides in the steamed ginseng, accounting for 39% and 19% of all ginsenosides, respectively.

Heating not only leads to thermal decomposition of ginsenosides, but also change theirs optical isomer types. Wang *et al*. [178] found that, after 2 h of steaming, as the steaming time increased, the level of 20(S)-Rg2 decreased, while the level of 20(R)-increased in American ginseng berry.

1.4.2 Acidic and alkaline cleavage of ginsenosides

In 1981, Han *et al*. [179] reported that ginsenoside Rb1, Re and Rg1 decomposed to produce artifact products by acidic treatment under physiological condition such as 37℃ incubation in 0.1 M HCl. Moreover, Bae *et al*. [180] found that the PPD-type ginsenosides were transformed to Rg3 or Δ^{20}-Rg3 after incubated at 60℃ in acidic conditions. Acidic cleavage not only transform ginsenosides, but also produce some other unidentified compounds due to the side reactions.

In 1987, Chen *et al*. [181] succeed in preparing ginsenoside Rh1 and protopanaxtriol by direct treatment of the peracetate ginsenoside Rg2 in alcoholic alkaline solutions. Afterwards, Im *et al*. [182] established a modified method to obtain protopanaxadiol and protopanaxatriol by complete hydrolysis of ginsenosides with sodium methoxide in dry pyridine solution. However, prolonged treatment with alkali yields some unidentified artifact compounds [182, 183].

1.4.3 Microbial transformation of ginsenosides

Some intestinal bacteria can hydrolysis ginsenosides and use the sugar moieties as carbon source. A number of researchers utilize microbes isolated from human intestine to transform ginsenosides. Kim *et al.* [184] reported that human intestine microflora transformed Rb1 and Re to cK and Rh1, relatively. Bae *et al.* [185] reported that ginsenoside Rc could be

effectively transformed to cK and protopanaxadiol by human fecal microflora. Most of the intestinal bacteria, including *Bacteroides* sp., *Eubacterium* sp. and *Bifidobacterium* sp. potently transformed Rc to cK via Rd.

Fungi are highly amenable to culture and suitable fungus might replace the need for human intestinal bacteria for transformation of ginsenoside. Microorganisms living in the environment of soil-cultivated ginseng might have a potential to effectively transform ginsenosides. Han *et al*. [186] reported that *Fusarium sacchari*, obtained from soil-cultivated ginseng, possess a potent capacity of transforming Rb1 to cK. Other fungi, such as *Aspergillus strictum*, *A. niger*, and *Fusarium moniliforme* could also transform the PPD-type ginsenosides to cK efficiently [187, 188].

Since most microorganism used to transform ginsenosides are not of a food-grade standard, many researchers, for rectifying this concern, reported that ginsenosides could be transformed by extracts from various edible microorganisms, such as *Bifidobacterium sp.* Int57, *Aspergillus niger*, *A. usamii* [189, 190]. Microorganism used to transform ginsenosides must need to fulfill two criteria: (1) potent to transform ginsenosides; (2) to be of a food-grade standard.

1.4.4 Enzymatic transformation of ginsenosides

Glycosidases, like β-glucosidase, β-galactosidase, α-rhamnosidase,

lactase, hesperidinase and naringinase, can be used to hydrolysis the sugar moieties in order to transform ginsenosides. Park *et al*. [191] reported that β-glucosidase from *Fusobacterium* K-60 transformed Rb1 to cK. As for transformation of the PPT-type ginsenosides, Yu *et al*. [192] reported that α-rhamnosidase from *Absidia* sp. 39 transformed Rg2 to Rh1. Ko *et al*. [193] reported that β-galactosidase from *A. orzyae*, lactase and hesperidinase from *Penicillium* sp., naringinase from *P. decumbens* transformed the PPT-type ginsenoside to Rg2, Rh1, Rg1, and F1, respectively.

1.5 Objective of the present research

Since both ginseng root and ginseng berry have been used to develop functional foods or tonics, a deep understanding of their bioactivity profiles and mechanisms is pretty suggestive. Although the anti-obesity and anti-diabetic effects of ginseng berry in ob/ob or db/db mice have been reported, the protective effect of crude saponin isolated from fermented ginseng berry in non-genetic-engineered obese mice induced with high-fat diets is not clear. Moreover, as the ginsenoside profiles are distinct from each other, the root and the berry might exhibit different a pattern of activities. Whether the root or the berry possesses a more potent protective effect against obesity in HFD-induced obese mice remains to be studied.

Now that the deglycosylated forms of ginsenoside are more easily absorbed and exert more potent bioactivities, it is necessary to transform ginsenosides before oral ingestion. Retarding the digestion and absorption of fats in the intestinal tract reduces energy harvest, which helps to prevent and improve obesity and diabetes. As described previously, ginsenosides can work as pancreatic lipase inhibitors. The present research is aimed to screen various strains of *A. niger* and *A. orzyae* in order to transform ginsenosides in ginseng root and ginseng berry, and compare the anti-obesity effects between the root and the berry in the aspects of inhibition on the activity of pancreatic lipase and regulation of body weight and lipid

metabolism in obese mice induced with HFD.

Chapter 2 Fermentation of ginseng root and ginseng berry

2.1 Introduction

Various transformation methods like heating [177], mild acid hydrolysis [194], alkaline cleavage [181], enzymatic hydrolysis [195] and microbial conversion [189] have been reported. However, heating, used to make red ginseng, produce inevitable and undesirable side reactions and cannot completely transform ginsenosides. The chemical methods sometimes are not safe and produce side reactions, including epimerization, hydration and hydroxylation. Enzymatic methods are also limited because they are complicated to operate and hard to use in foods. Due to these limitations, some researchers choose microorganism to transform ginsenosides. However, microorganisms used are often not of a food-grade standard. Many researchers have reported during the past few decades that ginsenosides can be successfully transformed by human intestinal bacteria [196-198]. However, these intestinal bacteria requires expensive medium and may exhibit low yield and poor productivity [199]. There are also other researchers utilizing microorganisms isolated from soil-cultivated ginseng to transform ginsenosides [186, 187]. Nevertheless, soil microorganisms can be applied to foods only if they have been proved to be safe.

In previous studies [189, 190], we have researched the transformation of ginsenosides by various microorganisms that have been safely used for foods consumption. Some strains of fungus also have strong saccharifying

power and have long been used to ferment traditional foods in Korea, China, Japan and other countries. In this work, we screened various strains of *Aspergillus niger* and *Aspergillus oryzae* for the transformation of ginsenosides and finally two strains were selected to ferment ginseng berry and ginseng root according to the transformation efficiency.

2.2 Material and methods

2.2.1 Materials and chemicals

The 4-year Korean ginseng (Panax ginseng C.A. Meyer, family Araliaceae) roots were purchased from Nokdu Market (Seoul Korea). Ginseng berry (the berry of Panax ginseng C.A. Meyer, family Araliaceae, harvested in early June, green color) was provided by Korean Genetic Pharm (Seoul, Korea). Standard ginsenosides such as Rb1, Rb2, Rd, and Rg1 were purchased from Biotech (Nanjing, China). Ginsenoside Re, Rg2, Rg3, F2, Rh1, Rh2, and cK were purchased from Cogon Biotech (Chengdu, China). HPLC grade acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ). Other chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise mentioned.

2.2.2 Fermentation of ginseng berry and ginseng root

Dried ginseng root and ginseng berry were powdered and mixed with stilled water in 1:10, 1:15 ratio, respectively, before being extracted at 80℃ for 3 h in water bath with shaking. The extract was filtered with 4 layers of gauze and the filtrate used as culture medium. Various strains of *A. niger* and *A. orzae* were from the Lab of Food Microbiology in Seoul National University and were cultured with PDA medium under aerobic conditions at 30℃ for several days. The spores were scraped from PDA plate and suspended in saline containing 0.005% Tween 80. After the concentrations

of the spores were determined with a hemocytometer, the spores were inoculated at a density of 10^6 spores/mL in the prepared medium. The culture broth was incubated at 30℃ for 96 h under aerobic conditions with shaking.

2.2.3 Preparation of crude saponin samples from fermented ginseng root and berry

Fermented culture broth was freeze-dried and extracted with water-saturated n-butanol at 80℃ for 1 h. After filtration with Whatman No. 41 filter paper (Kent, UK), the filtrate was mixed with appropriate amount of distilled water and stewed overnight. The upper phase was evaporated with a speed vacuum concentrator and the left solid content was suspended in diethyl ether and maintained at 46℃ for 1 h so as to remove the fat-soluble impurities. The produced crude saponins were used as samples for thin-layer chromatography (TLC) analysis, HPLC analysis and studies in the next chapters.

2.2.4 Analysis of ginsenosides using TLC

Silica gel 60 F254 plate of Merck KGaA (Darmstadt, Germany) was used to conduct TLC analysis. A mixture of chloroform, methanol, and water (65:35:10, v/v/v) was stewed overnight before the lower phase was used as the mobile phase. The plate was stained by spraying with H_2SO_4 in ethanol (10:90, v/v) and followed by heating.

2.2.5 Analysis of ginsenosides using HPLC

The crude saponins isolated from the fermented broth was analyzed by HPLC conducted with an Agilent HP 1090 Series instrument (Santa Clara, CA) and a diode array detector (DAD). A poroshell 120 EC-C18 column (3x 100 mm, 2.7 μm) from Agilent was used. The mobile phase consisted of water (A) and acetonitrile (B). The elution condition was optimized as follows: 0-16 min (19% B), 16-21 min (19-27% B), 21-30 min (27-29% B), 30-47 min (29-40% B), and 47-65 min (40-80% B). As for analysis of ginsenoside Rg2 and Rh1, an adjusted elution condition was used, which was 0-16 min (19% B), 16-35 min (19-30% B). The flow rate, injection volume, and detection wavelength were set as 0.5 mL/min, 20 μL, and 203 nm, respectively. Standard curves were constructed from the measured peak areas and the related concentration of ginsenosides. Various kinds of ginsenosides in the extract were identified by comparison of their retention times with those of ginsenoside standards. The contents of ginsenosides in each sample were calculated using standard curves.

2.3 Results and Discussion

2.3.1 Screening of fungus

Various strains of *A. niger* and *A orzyae* were screened using ginseng berry extract as the culture broth according to the transformation efficiency of ginsenosides tested by TLC. Ginsenoside cK and Rh1 are the main and final metabolites of the PPD-type and the PPT-type ginsenoside, respectively. Results showed that *A. niger* tended to transform the PPD-type ginsenosides to cK (Table 2-1) while *A. oryzae* tended to transform the PPT-type ginsenoside to Rh1 (Table 2-2). Different properties of ginsenoside transformation were showed between *A. niger* and *A. oryzae*, which might be explained by substrate specificity of glycosidase. Because ginseng root and ginseng berry contain the PPD-type ginsenoside, like Rb1, and the PPT-type ginsenoside, like Re, respectively, as their main ginsenosides, we finally chose *A. niger* FMB S494 and *A. orzyae* FMB S247 to ferment ginseng root and ginseng berry, respectively.

A. niger and *A. oryzae* are generally considered safe nontoxic fungi and play important roles in processing various fermented foods [200]. However, it was reported that certain strains of *A. niger* could produce ochratoxin and fumonisin while some strains of *A. oryzae* could produce aflatoxin and cyclopiazonic acid [201]. In this research, the selected *A. niger* FMB S494 and *A. orzyae* S247 do not produce mycotoxins [202, 203].

Table 2-1 The ginsenoside transforming ability of various strains of *A. niger* evaluated by TLC.

Strains	cK	Rh1
A. niger FMB S280	+	
A. niger FMB S018	+	
A. niger FMB S589	+	
A. niger FMB S547	+	
A. niger FMB S333	+	
A. niger FMB S493	+	
A. niger FMB S494	++++	+
A. niger FMB S497	++	
A. niger FMB S58	+	
A. niger FMB S10	+	
A. niger FMB S13	+	
A. niger FMB S23	+	
A. niger FMB S56	+	
A. niger FMB S00	+	
A. niger FMB S01	+	
A. niger FMB S07	+	
A. niger FMB S87	+++	
A. niger FMB S88		
A. niger FMB S90	+	
A. niger FMB S91		
A. niger FMB S94	+++	
A. niger FMB S002		
A. niger FMB S025	+++	

The number of “+” represents the relative abundance of relevant ginsenosides.

Table 2-2 The ginsenoside transforming ability of various strains of A. oryzae evaluated by TLC.

Strain	cK	Rh1
A. oryzae FMB S232		
A. oryzae FMB S234		
A. oryzae FMB S242		++
A. oryzae FMB S247	+	+++
A. oryzae FMB S250		+++
A. oryzae FMB S730	+	+
A. oryzae FMB S736		++
A. oryzae FMB S246	+	+
A. oryzae FMB S823		
A. oryzae FMB S847		
A. oryzae FMB S966		
A. oryzae FMB S967		
A. oryzae FMB S968		++
A. oryzae FMB S969		
A. oryzae FMB S971		
A. oryzae FMB S988		
A. oryzae FMB S989	+	++
A. oryzae FMB S990		++
A. oryzae FMB S991		++
A. oryzae FMB S992		+
A. oryzae FMB S993		
A. oryzae FMB S994		
A. oryzae FMB S995		+++
A. oryzae FMB S997		
A. oryzae FMB S001		
A. oryzae FMB S002		
A. oryzae FMB S004		
A. oryzae FMB S006		
A. oryzae FMB S007		++
A. oryzae FMB S107		
A. oryzae FMB S471		
A. oryzae FMB S59		+++
A. oryzae FMB S95		+++
A. oryzae FMB S98		

The number of “+” represents the relative abundance of relevant ginsenosides.

2.3.2 Transformation of ginsenosides in ginseng root

According to the results of TLC (Fig. 2-1) and HPLC (Fig. 2-2), the main ginsenosides in the unfermented ginseng root are Rb1, Rb2, Rc, Rd, Re, Rf and Rg1, which is in line with the previous study [91]. Bands of ginsenosides ascended on the TLC plate after fermentation, which showed that ginsenosides were transformed to their less polar forms. The band of cK markedly appeared on the top of the column of fermented ginseng root. After fermentation by *A. niger*, according to the quantitative analysis with HPLC (Fig. 2-3), the PPD-type ginsenosides such as Rb1, Rb2, Rc and Rd decreased from 9.3% to 0, 3.7% to 0, 2.5% to 0 and 5.0% to 0, respectively. Rg3, F2, Rh2 and cK increased from 0.9% to 4.9%, 0 to 1.5%, 0 to 2.9% and 0 to 9.1%, respectively. The PPT-type ginsenosides such as Re, Rg1 and Rf decreased from 7.0% to 2.9%, 6.8% to 4.6% and 4.3% to 2.4%, respectively. Rg2 and Rh1 increased from 0 to 1.9% and 0 to 2.6%, respectively.

In summary, *A. niger* FMB S494 effectively transformed the PPD-type ginsenosides to cK and moderately transformed the PPT-type ginsenosides to Rh1. Moreover, *A. niger* was more apt to transform the PPD-type ginsenosides than the PPT-type with a dramatic increased content of cK to 9.1% (Fig. 2-4). Ginsenoside cK was reported to possess many biological and pharmaceutical effects such as anti-inflammation [204], anti-obesity [138], anti-diabetes [205], anti-cancer [206], neuroprotection and cognition

enhancement [207].

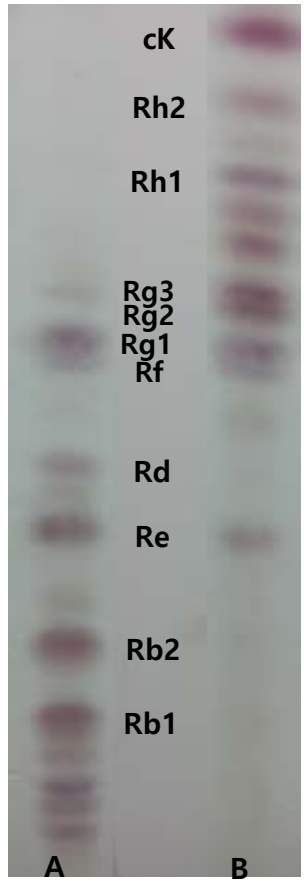


Fig. 2-1 TLC profiles of ginsenosides of crude saponins isolated from unfermented and *A. niger*-fermented ginseng root.

A. unfermented ginseng root, B, *A. niger*-fermented ginseng root.

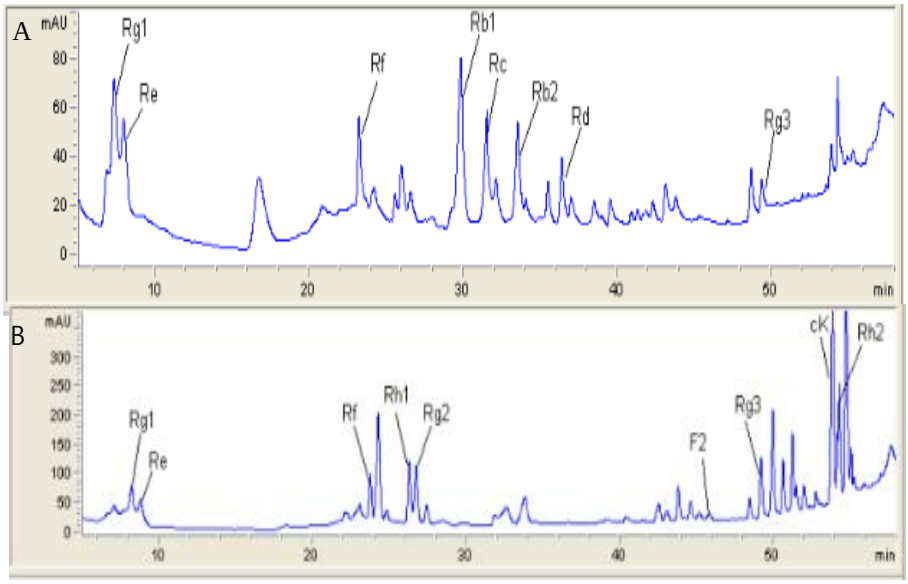
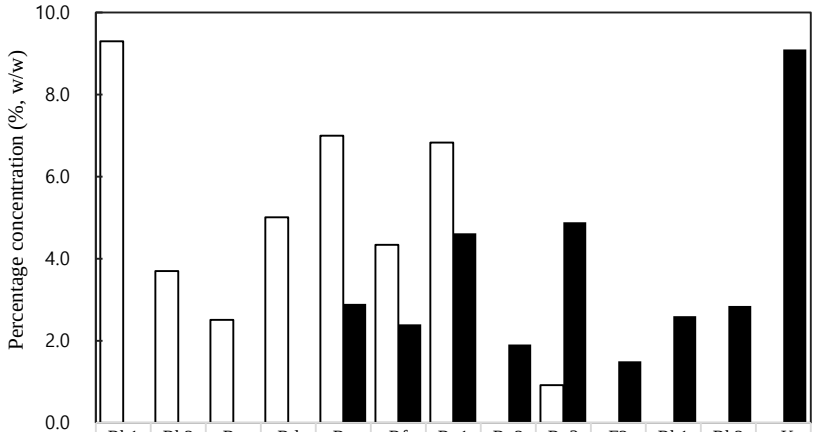


Fig. 2-2 HPLC chromatograms of ginsenosides detected from the crude saponins isolated from unfermented and *A. niger*-fermented ginseng root.

A, unfermented ginseng root; B, *A. niger*-fermented ginseng root.



	Rb1	Rb2	Rc	Rd	Re	Rf	Rg1	Rg2	Rg3	F2	Rh1	Rh2	cK
□GR	9.3	3.7	2.5	5.0	7.0	4.3	6.8	0	0.9	0	0	0	0
■FGR	0	0	0	0	2.9	2.4	4.6	1.9	4.9	1.5	2.6	2.9	9.1

Fig. 2-3 Ginsenoside contents in crude saponins isolated from unfermented and *A. niger*-fermented ginseng root measured by HPLC.

Percentage concentration = weight of ginsenoside/ weight of sample×100%

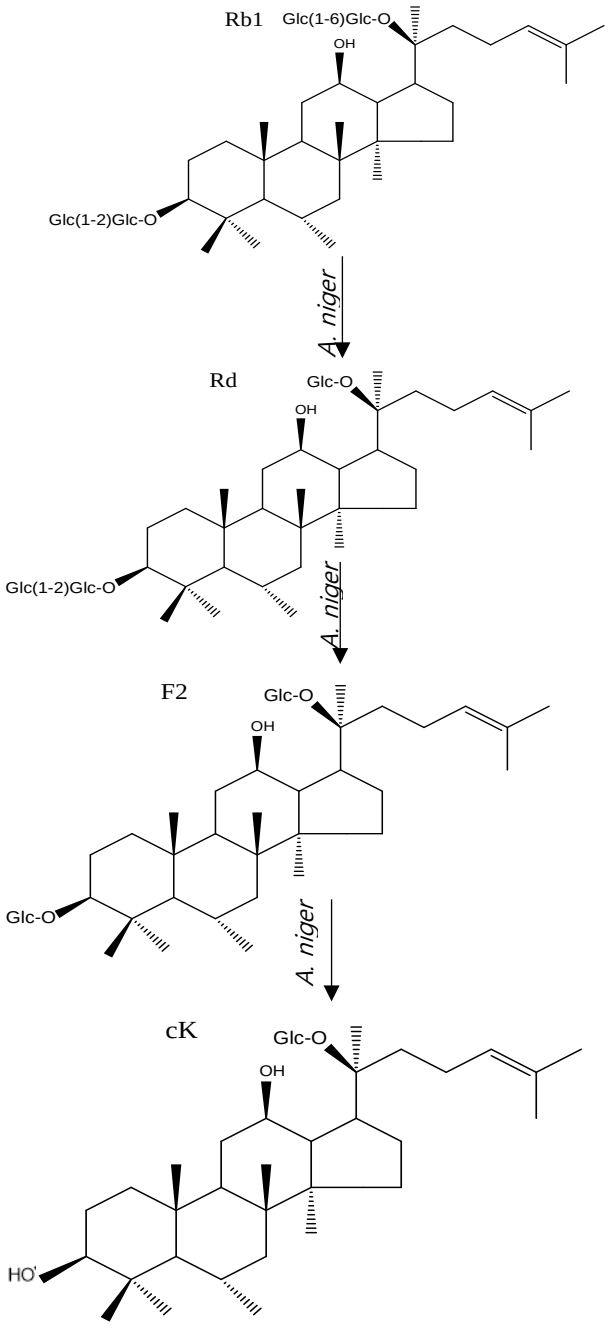


Fig. 2-4 Proposed main transformation pathway of ginsenoside Rb1 by *A. niger* FMB S494.

2.3.3 Transformation of ginsenosides in ginseng berry

The PPT-type ginsenoside Re is the most plentiful ginsenoside in ginseng berry. Ginsenoside Rb1, Rb2, and Rd are also contained as the main PPD-type ginsenosides in the berry. After fermentation, the ginsenoside profile was analyzed by TLC (Fig. 2-5) and HPLC (Fig. 2-6). The percentage weight of various ginsenosides in the crude saponin isolated from the fermented berry was determined by HPLC (Fig. 2-6).

In term of TLC results (Fig. 2-5), the bands of ginsenosides on the plate went up after fermentation. Furthermore, the band of Rh1 markedly emerged on the top of the column of fermented ginseng berry. These show that the major ginsenosides have been transformed to their less polar forms.

After fermentation by *A. oryzae* FMB S247, for the main PPT-type ginsenosides, Re decreased from 21.2% to 0%, Rg1 increased from 7.9% to 19.1% and Rh1 increased from 0.2% to 7.8%. As for the PPD-type ginsenosides, Rb1 decreased from 2.0% to 0.5%, Rb2 decreased from 4.9% to 2.5%, and Rd decreased from 9.9% to 1.3%; F2 increased from 0 % to 9.2%, and cK increased from 0% to 1.3% (Fig. 2-7). Thus it can be seen that *A. oryzae* transformed PPD-type ginsenosides mainly to F2 and slightly to cK. Furthermore, ginsenoside Rg3 was also produced during sterilization with autoclave (data not shown). As for the PPT-type ginsenosides, Re was largely transformed to Rh1 via Rg1 (Fig. 2-8). Thus, it is apparent that *A.*

oryzae may be more prone to transform the PPT-type ginsenosides, with a high yield of ginsenoside Rh1, while the PPD-type ginsenoside transformation was stopped at ginsenoside F2, a less deglycosylated form.

Taken together, ginsenosides in ginseng berry were potently transformed into their less polar forms. Ginseng berry fermented by *A. oryzae* FMB S247 contains abundant Rg1 and Rh1, which indicates that the *A. oryzae* tended to transform the PPT-type ginsenoside to Rh1. Rh1 was also reported to show anti-cancer [208], anti-allergy [209], anti-obesity [152] and protective activities on central nervous system [210].

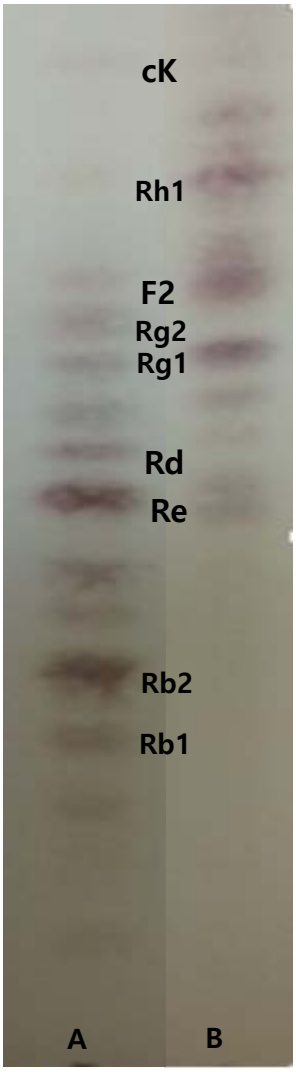


Fig. 2-5 TLC profiles of ginsenosides in crude saponins isolated from unfermented and *A. oryzae*-fermented ginseng berry.

A. unfermented ginseng berry, B, *A. oryzae*-fermented ginseng berry.

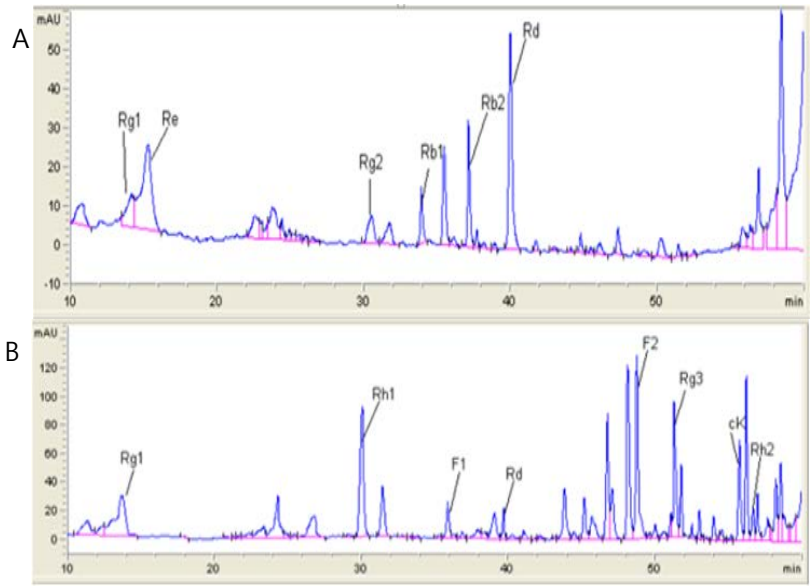
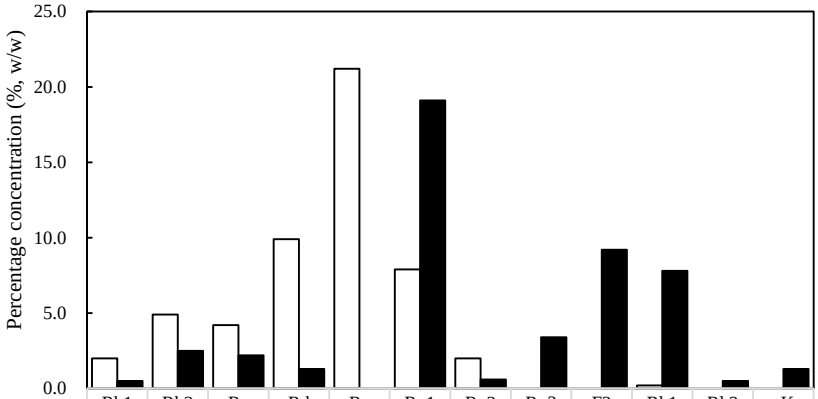


Fig. 2-6 HPLC chromatograms of ginsenosides detected from unfermented ginseng berry and *A. oryzae*-fermented ginseng berry.

A. unfermented ginseng berry, B, *A. oryzae*-fermented ginseng berry.



	Rb1	Rb2	Rc	Rd	Re	Rg1	Rg2	Rg3	F2	Rh1	Rh2	cK
□GB	2.0	4.9	4.2	9.9	21.2	7.9	2.0	0	0	0.2	0	0
■FGB	0.5	2.5	2.2	1.3	0	19.1	0.6	3.4	9.2	7.8	0.5	1.3

Fig. 2-7 Ginsenoside contents of crude saponins isolated from unfermented and *A. oryzae*-fermented ginseng berry measured by HPLC.

Percentage concentration = weight of ginsenoside/ weight of sample×100%

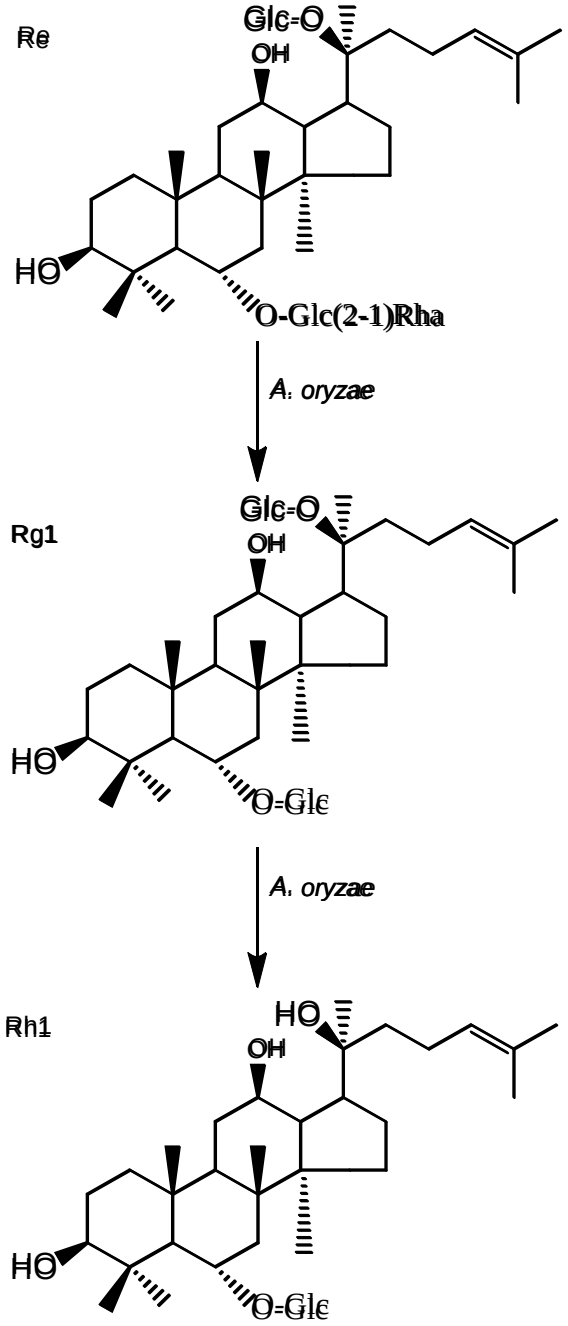


Fig. 2-8 Proposed main transformation pathway of ginsenoside Re by *A. oryzae* FMB S247.

2.4 Summary

To transform ginsenosides, 23 trains of *A. niger* and 34 strains of *A. orzyae* were screened. It is found that *A. niger* is more inclined to transform the PPD-type ginsenoside to cK, and that *A. orzyae* tends to transform the PPT-type ginsenoside to Rh1. Ginseng root and ginseng berry have distinct ginsenoside profiles. The main ginsenoside in the root is the PPD-type, like Rb1, while the main ginsenoside in the berry is the PPT-type, like Re. Therefore, we selected *A. niger* FMB S494 and *A. oryzae* FMB S247 to ferment ginseng root and ginseng berry, respectively. These two strains do not produce mycotoxins and can be considered safe. After fermentation, *A. niger* FMB S494 effectively transformed the PPD-type ginsenosides, like Rb1and Rb2 and Rd to cK in the root, with a high yield of cK (9.1%), while *A. oryzae* FMB S247 effectively transformed the PPT-type ginsenosides, like Re, to Rh1 via Rg1 in the berry, with a high yield of Rg1 (19.1%) and Rh1 (7.8%). Crude saponin isolated from the fermented ginseng root and berry are used in the subsequent studies.

Chapter 3 Effects of various ginsenosides and crude saponins isolated from ginseng root and berry on the activity of pancreatic lipase

3.1 Introduction

Obesity is considered to increase the risk of various diseases, especially heart disease, type 2 diabetes and certain types of cancer, and impair quality of life [211-213]. Retarding the digestion and absorption of carbohydrates and fats in the gastrointestinal tract reduces energy harvest, which helps to prevent and improve obesity and diabetes. Plenty of bioactive components from medicinal herbs have been reported to work as glucosidase inhibitors [214-216] or lipase inhibitors [217-219] and thereby prevent excess energy intake.

Pancreatic lipase, released by the pancreas, is an enzyme that converts triglyceride into free fatty acids and glycerol before absorption in the digestive system [220]. Ginsenoside Rb1, Rb2, Rc, Rd, and ginseng extracts have been shown to suppress the lipase activity [89, 90, 92, 221]. HFD-fed mice and rats supplemented with ginseng extracts also showed increased amount of feces and fecal lipid content [83, 93], which indicated that ginseng or ginsenosides might prevent or improve obesity via increasing the excretion of fat into feces. However, the inhibitory effect on the lipase activity of other ginsenosides, especially the deglycosylated forms, have not yet been reported.

In this work, we observed and compared the effects of various ginsenosides like the PPD-type - Rb1, Rb2, Rc, Rd, Rg3, F2, Rh2, cK, and

the PPT-type - Re, Rf, Rg1, Rg2 and Rh1. In addition, comparisons and analyses are made on the effects of the crude saponins isolated from fermented ginseng root and fermented ginseng berry along with the unfermented ones since fermentation changed their ginsenoside profiles.

3.2 Materials and Methods

3.2.1 Materials and chemicals

A. niger FMB S494 and *A. oryzae* FMB S247 that do not produce mycotoxin were from the Lab. of Food Microbiology, Seoul National University. Ginseng roots and ginseng berries were fermented with the *A. niger* and *A. oryzae*, respectively, as described previously. Crude saponins were isolated using the method described in Chapter 2. The NEFA assay kit was purchased from Wako (Osaka, Japan) while porcine pancreatic lipase (L3126), orlistat (O4139) and dimethylsulfoxide (DMSO) were purchased from Sigma (St. Louis, MO). Finally, triolein was purchased from Avention (Incheon, Korea).

3.2.2 Activity assay of pancreatic lipase

Crude pancreatic lipase powder was suspended in sterilized distilled water (0.1 g/mL) and centrifuged at 12000×g for 10 min. The supernatant was used as enzyme solution. Tris-HCl buffer (0.1 M, pH=8) was used as assay buffer. Triolein was dissolved in acetone at a concentration of 5% and the mixture was used as substrate solution. Various ginsenoside and the saponins isolated from unfermented and fermented ginseng root or berry were dissolved in DMSO. Afterwards, 80 μL of the assay buffer, 5 μL of the enzyme solution, and 10 μL of various samples were added to a 96-well plate. The reaction was started by adding 5 μL of the substrate solution

at 37℃ with 550 rpm shaking. After 30 min, the reaction was stopped by putting the plate on ice for 10 min. Then 7 μL of the reaction mixture was transferred to another 96-well plate. The generated amount of oleic acid in the reaction mixture was determined by using a NEFA assay kit according to the manufacture's instruction.

3.2.3 Analysis of triglyceride in feces

After fasted for 12 h and then administrated with the root saponin or the berry saponin, ICR mice were allowed high-fat food *ad libitum* for 10 h. The feces of mice were collected for analysis of triglyceride. Briefly, 0.6 g of feces was mixed with 600 μL of PBS, 1600 μL chloroform and 800 μL methanol, and allowed to stand overnight at 4℃. Afterward, 480 μL of 0.88% KCl solution was added and the mixture was vortexed vigorously, and then centrifuged at 1000 × *g* for 15 min. The lower centrifuged layer was evaporated under a hood and the residue was dissolved in 100 μL of isopropanol. Levels of triglyceride were determined by using appropriate kits from Asanpharm.

3.2.4 Statistical analysis

Results were expressed as mean ± standard deviation. Differences were tested by using one-way ANOVA and applying the least significant range tests. Statistical analyses used the SPSS statistical package (Chicago, IL). The significance level of the test results was set to $p < 0.05$.

3.3 Results and Discussion

3.3.1 Effects of various ginsenosides on pancreatic lipase activity

Effects of PPD-type ginsenoside Rb1, Rb2, Rc, Rd, Rg3, F2, Rh1, and cK on the lipase activity were tested *in vitro* (Fig. 3-1A). Results showed that ginsenosides Rb1, Rb2, Rc, Rd, Rg3, and cK have a tendency to inhibit the lipase activity. Among them, ginsenoside Rb1, Rd, Rg3, and cK significantly inhibited 43%, 47%, 75% and 55% of the lipase activity, respectively, at the concentration of 100 μg/mL. Ginsenoside Rh2 at the tested concentration showed no effect while ginsenoside F2 significantly increased 42% of the PL activity at the concentration of 200 μg/mL. Interestingly, ginsenoside Rg3 showed the most effective inhibitory effect while its metabolite, ginsenoside Rh2, showed no effect. Overall, ginsenosides of deglycosylated form such as Rg3 and cK showed more effective inhibitory effect than the precursor ginsenoside, like Rb1, Rb2, Rc, and Rd.

With respect to the PPT-type ginsenosides (Fig. 3-1B), Re and Rh1 showed no effect while ginsenoside Rg1 significantly inhibited 30% of PL activity at the concentration of 50 μg/mL. Both ginsenoside Rf and Rg2 showed the tendency to increase the lipase activity, but only ginsenoside Rf significantly enhanced it.

These results indicate that the effects of ginsenosides on the pancreatic lipase vary with each individual ginsenoside. Overall, the PPD-type ginsenosides show more effective inhibitory effect on the lipase activity compared with the PPT-type ginsenosides.

Because Rg3 inhibited almost 70% of the lipase activity at the concentration of 50 μg/mL, its inhibitory effect was furtherly tested at much lower concentrations. As shown in Fig. 3-2A, the minimum effective concentration of Rg3 was 6.25 μg/mL, where about 40% of the lipase activity was inhibited. The half maximal inhibitory concentration (IC_{50}) of Rg3 was between 6.25 μg/mL and 12.5 μg/mL.

Orlistat, an anti-obesity drug, was used as a positive control (Fig. 3-2B). Orlistat also works as a lipase inhibitor. The mechanism of ginsenosides on the lipase activity might be different from that of orlistat which inhibits lipase activity by covalently binding to the serine residue of the active site [222]. It was supposed that ginsenosides might attach to the surface of triglyceride droplet and disturb the access of pancreatic lipase to the substrate [90].

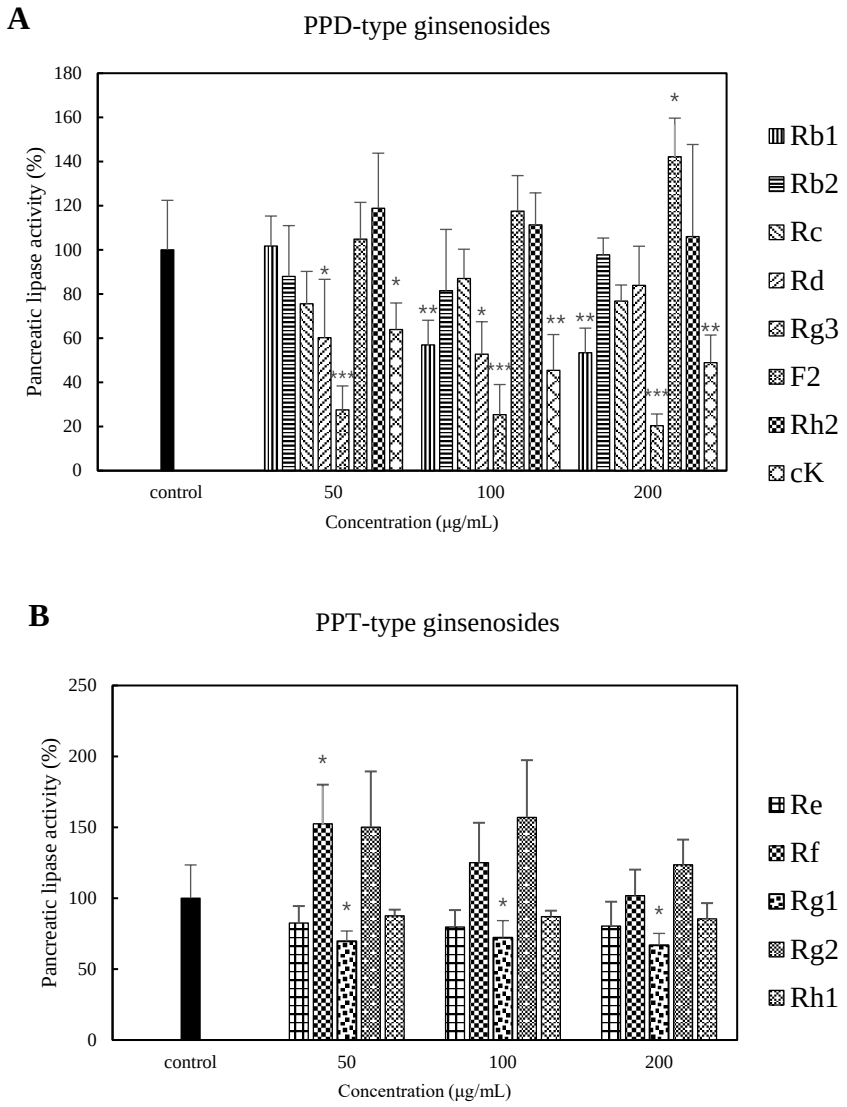


Fig. 3-1 Effects of various ginsenosides on the activity of pancreatic lipase.

A, PPD-type ginsenosides; B, PPT-type ginsenosides. Pancreatic lipase activity = (Abs after treatment/Abs of control) ×100%. *p<0.05, **p<0.01, ***p<0.001 vs. control (n=3).

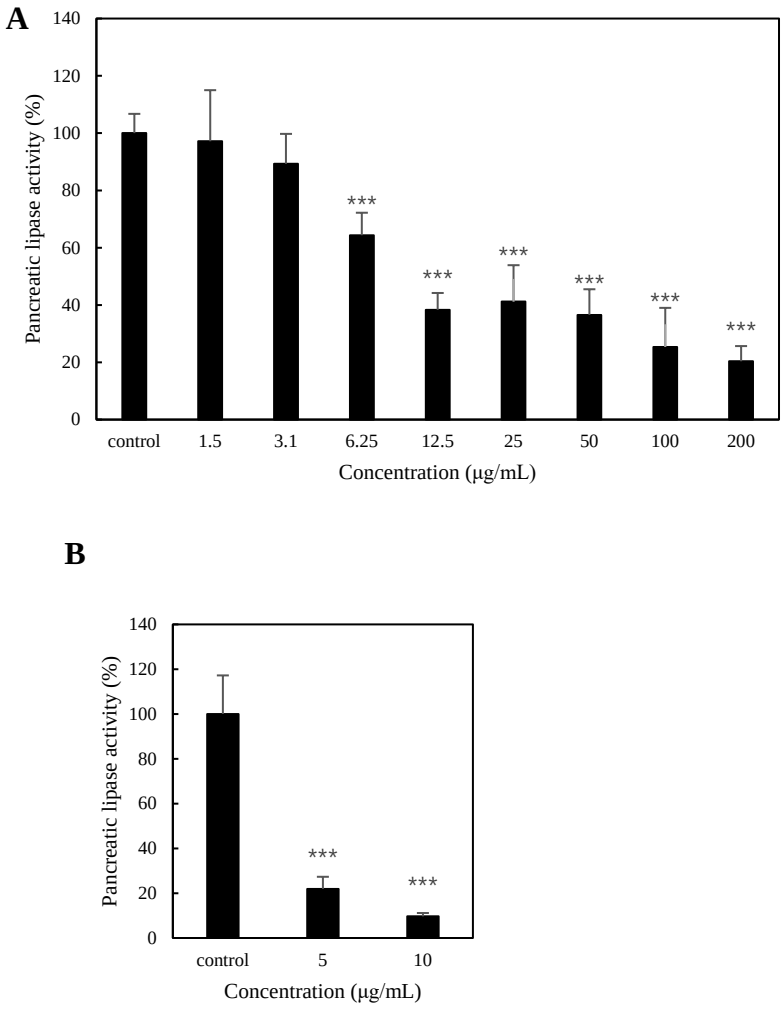


Fig. 3-2 Inhibitory effects of ginsenoside Rg3 and orlistat on the activity of pancreatic lipase.

A, ginsenoside Rg3; B, orlistat. Pancreatic lipase activity = (Abs after treatment/Abs of control) ×100%. ***p<0.001 vs. control (n=3).

3.3.2 Effects of saponins from ginseng root and berry on pancreatic lipase activity

The effects of the saponins isolated from the unfermented and fermented ginseng root or berry on the lipase activity were also tested. In terms of the root, both the unfermented and the fermented saponins significantly inhibited lipase activity (Fig. 3-3A). The unfermented root saponin and the fermented root saponin inhibited 52% and 86% of the lipase activity at the concentration of 1 mg/mL, respectively. Fermentation enhanced the inhibitory effect of the root saponin, which might be attributed to the increased contents of cK and Rg3.

With respect to the berry, unfermented berry saponin showed no effect at the tested concentration while the fermented one significantly inhibited 48% of the lipase activity at the concentration of 0.25 mg/mL (Fig. 3-3B). Fermentation dramatically enhanced the inhibitory effect of the berry saponin, which might be attributed to the increased contents of ginsenoside Rg1, Rg3, and cK.

At the concentration of 1 mg/mL, fermented root saponin inhibited nearly 90% of the lipase activity while fermented berry saponin only inhibited about 60% of the activity. Since the PPD-type ginsenosides are more effective than the PPT-type and the root contains more PPD-type ginsenosides like Rg3 and cK than the berry, saponin isolated from

fermented root was more potent than the that isolated from berry with regards to the inhibitory effect on the lipase activity.

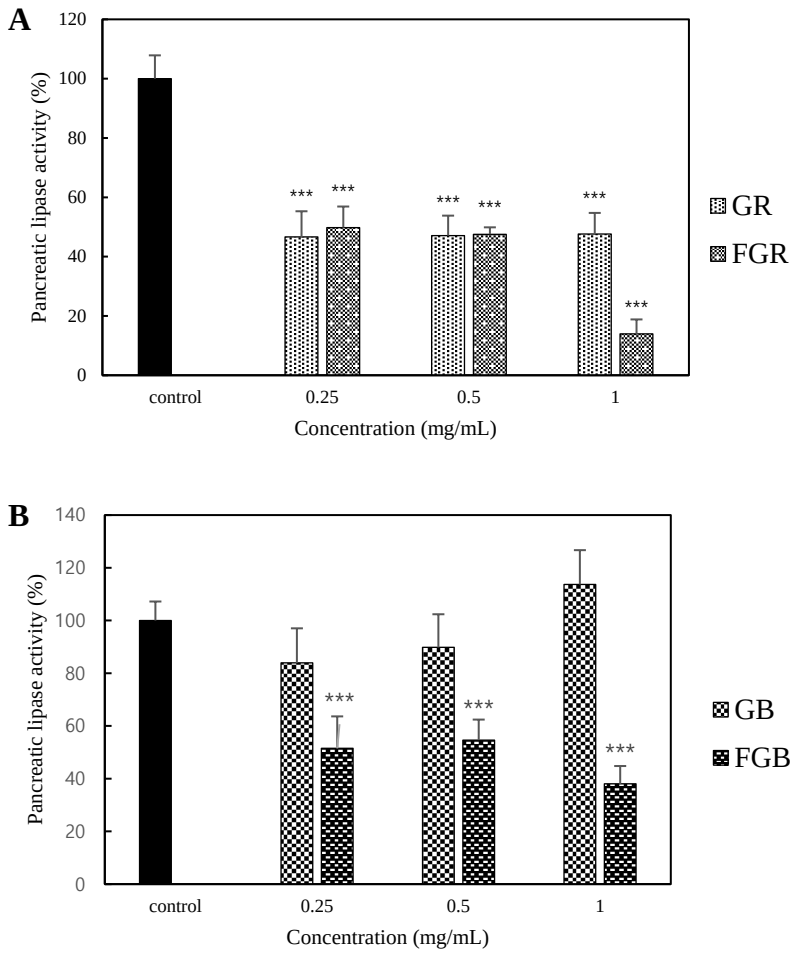


Fig. 3-3 Inhibitory effects of crude saponins separated from ginseng root or ginseng berry on the activity of pancreatic lipase.

A, GR, ginseng root; FGR, fermented ginseng root; B, GB, ginseng berry; FGB, fermented ginseng berry. Pancreatic lipase activity = (Abs after treatment/Abs of control) ×100%. ***p<0.001 vs. control (n=3).

3.3.3 Contents of triglyceride in the feces

The content of triglyceride in the feces of HFD-fed mice treated with the root saponin was significantly higher than that of mice in the vehicle group ($p<0.05$), while the berry saponin showed no effect (Fig. 3-4), which indicates that the root saponin more potently inhibits the activity of pancreatic lipase and thereby retards fat absorption *in vivo*.

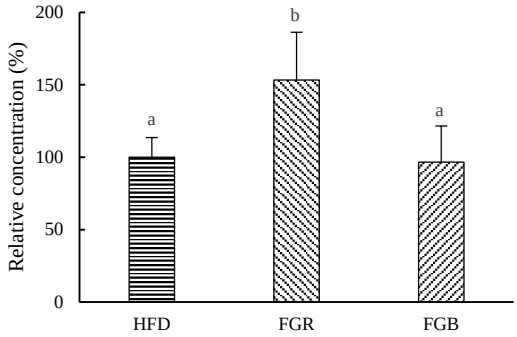


Fig. 3-4 Triglyceride contents in the feces of mice treated with crude saponins isolated from fermented ginseng root and berry.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$.

3.4 Summary

A large number of research has indicated that ginseng and ginsenoside exert anti-obesity effects in HFD-fed mice or rats [83, 106, 223, 224]. Ginsenoside Rb1 is reported to significantly ameliorate hepatic fat accumulation in HFD-induced obese rats [88]. Ginsenoside Re is reported to markedly lower blood glucose and triglyceride levels and protect against hepatic steatosis in HFD-fed mice[99]. The cK is reported to possess hypoglycemic and insulin-sensitizing capabilities on type 2 diabetes induced by HFD and streptozotocin [205]. Ginsenoside Rh1 is reported to attenuate obesity by inhibiting adipocyte differentiation and inflammation [152].

Since dietary lipid is the major source of superfluous energy, the inhibition of pancreatic lipase activity and digestion of triglycerides in the intestine might be very important for the anti-obesity effect of ginseng products. The development of obesity is characterized by a long-term imbalance between energy intake and energy expenditure and excess food intake is considered as the primary cause [76]. The inhibitory effect on the lipase activity might be a key mechanism of the anti-obesity effect of ginseng and some ginsenosides.

In conclusion, the inhibitory effect on pancreatic lipase activity of various common ginsenosides was evaluated. The results show that

ginsenoside Rg3 is the most potent one. Ginsenoside Rg3 can be generated from ginsenoside Rb1 or Rb2 by heating and red ginseng is known to be rich in ginsenoside Rg3. In addition, the PPD-type ginsenosides are more potent than the PPT-type ginsenosides. Ginsenoside cK, a regular metabolite of the PPD-type ginsenosides and reputed to exert various biological activities [205, 225], is found to more remarkably suppress the lipase activity than its precursors. Moreover, crude saponin isolated from the root showed more potent effect than that of the berry and fermentation markedly enhances the inhibitory ability of the root and berry saponins on the lipase activity, which might be attributed to the increased contents of ginsenoside Rg1, cK and Rg3.

Chapter 4 Anti-obesity effects of crude saponins isolated from fermented ginseng root and berry

4.1 Introduction

Panax ginseng root and ginseng berry have a distinct ginsenoside profile. The representative ginsenosides in ginseng root are the PPD-type while that in ginseng berry are the PPT-type. Both the root and the berry extracts have been reported to exhibit anti-obesity and anti-diabetic effects in murine model [83, 226-228]. Anoja *et al*. [226] showed that the berry extract reduced blood glucose levels, food intake and body weight, and increased energy expenditure in ob/ob mice. Dey *et al*. [227] compared the anti-hyperglycemic and anti-obesity effect between the root and the berry, and found that the berry exhibited a more potent hypoglycemic activity, and that only the berry showed marked anti-obesity effects in ob/ob mice. In their study, however, ginseng root and ginseng berry extracts were administrated to mice intraperitoneally, which ignored the activities of the ginseng components in the gastrointestinal tract. It was reported that ginseng and ginsenosides could work as pancreatic lipase inhibitors and thereby delay the digestion and absorption of lipids [90]. As described in the previous chapter, both saponins from the fermented ginseng root and berry significantly suppressed the activity of pancreatic lipase. Moreover, the bioavailability of ginsenosides through intraperitoneal administration is far higher than that through oral administration. In addition, because the mice (ob/ob) used in that study were genetically engineered, the protective effects of ginseng berry against obesity in normal mice fed a HFD is not

clear. Therefore, whether ginseng root, or ginseng berry exerts a more potent activity remains unclear.

The aim of this study is to evaluate and compare the effects of crude saponin from fermented ginseng root and berry on obesity, and to elucidate their distinct mechanisms in mice fed a HFD.

4.2 Materials and methods

4.2.1 Materials and chemicals

A. niger FMB S494 and *A. oryzae* FMB S247 that do not produce mycotoxin were from the Lab. of Food Microbiology, Seoul National University. Ginseng roots and ginseng berries were fermented with the *A. niger* and *A. oryzae*, respectively, as described previously. Crude saponins were isolated using the method described in Chapter 2.

4.2.2 Animals and diets

Male C57BL/6 mice (5 weeks old), purchased from Central Lab. Animal (Seoul, Korea), were housed under a 12 h light/12 h dark cycle in a controlled room at a temperature of 23 ± 3°C and a humidity of 50% ± 10%. After acclimating to the facility for 1 week, the mice were randomly divided into 4 groups (n = 10) and fed a low-fat diet (LFD; 10% of the total calories from fat, Table 4-1), a high-fat diet (HFD; 45% of the total calories from fat, Table 4-1), or high-fat diet supplemented with crude saponin separated from the fermented root or berry for 16 weeks. All the mice were allowed food and water *ad libitum*. Body weight, fasting blood glucose and food intake were determined once every 2 weeks. Before blood glucose was determined, mice were fasted for 12 h. After consuming an LFD or HFD for 16 weeks, the mice underwent 12 h of fasting prior to being anaesthetized with zoletil™ 50 (Virbac, Carros, France) and rompun®

(Ansan, Korea) and then were dissected. Blood samples were collected by heart punctures. Livers and epididymal fat pads were removed and stored at −80°C for subsequent analyses. All procedures relating to the animals and their care were approved by the Institutional Animal Care and Use Committee of Seoul National University.

Table 4-1 Formula of low fat diet and high fat diet.

Formula	LFD (10% calorie from fat, g/Kg)	HFD (45% calorie from fat, g/Kg)
Casein	210.0	245.0
L-Cystine	3.0	3.5
Corn Starch	280.0	85
Maltodextrin	50.0	115.0
Sucrose	325.0	200.0
Lard	20.0	195.0
Soybean Oil	20.0	30.0
Cellulose	37.15	58.0
Mineral Mix, AIN-93G-MX (94046)	35.0	43.0
Calcium Phosphate, dibasic	2.0	3.4
Vitamin Mix, AIN-93-VX (94047)	15.0	19.0
Choline Bitartrate	2.75	3.0
Yellow Food Color	0.1	0
Blue Food Color	0	0.1

4.2.3 Histopathologic evaluation

After mice were sacrificed, the samples of live and epididymal fat pads were fixed with formalin solution, stained with hematoxylin and eosin, and viewed with an optical microscope.

4.2.4 Biochemical analyses

Plasma levels of triglyceride (TG), total cholesterol (TC), HDL-C and activity levels of AST and ALT were determined by with test kits obtained from Asanpharm (Seoul, Korea). The levels of free fatty acid were determined with the NEFA assay kit. LDL-C levels were calculated with the formula LDL-C = TC− HDL-C – TG×0.2. Plasma insulin levels were determined with the mouse insulin ELISA kit from Shibayagi (Shibukawa, Japan), and plasma adiponectin levels were determined with the ELISA kit from Cloud-clone (Houston, TX).

4.2.5 Hepatic lipid analyses

Total lipid was extracted from mouse liver by using the Folch method [229] with some modifications. Approximately 25 mg of liver was mixed with 20 fold of phosphate buffered saline (PBS) and homogenized. The protein concentration of the homogenates was adjusted to 1 mg/mL using BCA assay kit (Berkeley, CA). Subsequently, 300 μL of the diluted homogenate was mixed with 800 μL chloroform and 400 μL methanol, and allowed to stand overnight at 4℃. Afterward, 240 μL of 0.88% KCl solution

was added and the mixture was vortexed vigorously and then centrifuged at 1000 × *g* for 15 min. The lower layer was evaporated under a hood and the residue was dissolved in 100 μL of isopropanol. Levels of TG and TC were determined using appropriate kits from Asanpharm.

4.2.6 Real-time polymerase chain reaction

Total RNA was extracted from the liver and adipose tissue with an RNA extraction kit purchased from Takara Bio (Kusatsu, Japan) and RNeasy® Lipid Tissue Mini Kit from Qiagen (Venlo, Netherlands), respectively. The concentration of RNA was measured with the Micro Spectrophotometer from Allsheng (Hangzhou, China), and 0.5 μg of total RNA from each sample was reverse-transcribed to cDNA with a cDNA synthesis kit from Takara Bio. Relative quantifications of gene transcripts were completed with SYBR premix from Takara Bio using the Applied Biosystems 7500 system. Relative mRNA levels were normalized to the *Gapdh* mRNA level and expressed as values of relative expression compared to that of the HFD group. The primers used in this study are listed in Table 4-2.

Table 4-2 Sequences of primers used in RT-PCR.

Gene	Forward (5'-3')	Reverse (5'-3')	Ref.
Acc	ATG GGC GGA ATG GTC TCT TTC	TGG GGA CCT TGT CTT CAT CAT	[230]
Acsl1	CAC TTC TTG CCT CGT TCC AC	GTC GTC CCG CTC TAT GAC AC	[231]
Adgre1	TCC AGC ACA TCC AGC CAA AGC	CCT CCA CTA GCA TCC AGA AGA AGC	[152]
ApoB100	TGA ATG CAC GGG CAA TGA	GGC ATT ACT TGT TCC ATG GTT CT	[232]
Atgl	CTC CGA GAG ATG TGC AAA CA	CAG TTC ACA CTG CTC AGA CA	[233]
Cd36	ACT TGG GAT TGG AGT GGT GAT GT	AGA TGT AGC CAG TGT ATA TGT AGG CTC	[234]
Cd68	TTC AGG GTG GAA GAA AGG TAA AGC	CAA TGA TGA GAG GCA GCA AGA GG	[152]
Cebpa	GAA CAG CAA CGA GTA CCG GGT A	GCC ATG GCC TTG ACC AAG GAG	[152]
Dgat2	CTT CCT GGT GCT AGG AGT GG	GCC AGC CAG GTG AAG TAG AG	[235]
Fabp1	GAA CTC ATT GCG GAC CAC TT	CAT CCA GAA AGG GAA GGA CAT	[236]
Fabp4	TGA TGC CTT TGT GGG AAC CT	GCA AAG CCC ACT CCC ACT	[152]
Fas	CTT CGC CAA CTC TAC CAT GG	TTC CAC ACC CAT GAG CGA GT	[237]
Fatp5	GAA TCG GGA GGC AGA GAA CT	AGC GGG TCA TAC AAG TGA GC	[238]
Gapdh	ACC ACA GTC CAT GCC ATC AC	TCC ACC ACC CTG TTG CTG TA	[239]
Gpat	GGT AGT GGA TAC TCT GTC GTC CA	CAG CAA CAT CAT TCG GT	[240]
Hmgcr	GGG CCC CAC ATT CAC TCT T	GCC GAA GCA GCA CAT GAT CT	[241]
Hsl	CTT CCT GCA AGA GTA TGT ACA G	TGG AGG TGA GAT GGT GAC TG	[242]
Il1b	AAC CTG CTG GTG TGT GAC GTT C	CAG CAC GAG GCT TTT TTG TTG T	[152]
IL6	CCG CTA TGA AGT TCC TCT CTG C	ATC CTC TGT GAA GTC TCC TCT CC	[152]
Ldlr	CCA CTT CCG CTG CAA ATC AT	TCA TGG GAG CCG TCA ACA C	[243]
Lpl	ATC CAT GGA TGG ACG GTA ACG	CTG GAT CCC AAT ACT TCG ACC A	[244]
Lrp1	GAC CAG GTG TTG GAC ACA GAT G	AGT CGT TGT CTC CGT CAC ACT TC	[245]
Pparg	CCA GAG CAT GGT GCC TTC GCT	CAG CAA CCA TTG GGT CAG CTC	[152]
Scd1	CGA GGG TTG GTT GTT GAT CTG	ATA GCA CTG TTG GCC CTG GA	[246]
Srebp1	GTG AGC CTG ACA AGC AAT CA	ACC AAG CCA GCA AAT ACA CC	[237]
Tnfa	TCT TCT CAT TCC TGC TTG TGG	GGT CTG GGG CAT AGA ACT GA	[152]

4.2.7 Statistical analysis

The differences among groups were examined with one-way ANOVA followed by the least significant range tests. Statistical analyses were done with the SPSS statistical package (Chicago, IL). The significance level of the test results was set at $p < 0.05$.

4.3 Results and discussion

4.3.1 Effects on food intake and body weight

The HFDs are considered to cause chronic inflammation in the hypothalamus and passivate leptin signaling, mediating sustained appetite enhancement [79]. Ginsenoside Rb1 was reported to reduce the levels of inflammatory markers and negative regulators of leptin signaling in the hypothalamus and restore the anorexic effect of leptin in HFD-fed mice [80]. Both the PPD-type and the PPT-type ginsenosides were reported to decrease orexigenic NPY and increase anorexigenic cholecystokinin in HFD-fed rats [81]. Moreover, many researchers have showed that ginseng extract could improve leptin resistance and diminish excessive energy intake in HFD-induced obese mice or rats [247].

In this study, the body weight of mice in the FGR and FGB groups were 29.7±1.9 g and 31.9±4.4 g, respectively, which are significantly lower than that of mice in the HFD group (36.2±4.4 g, $p<0.05$). In particular, mice in the FGR group showed the lowest weight gain (Fig. 4-1). In addition, food intake was also reduced in the FGR and FGB groups. Food effect ratio, body weight gain per unit food intake, was markedly lower in the FGR group than that in the HFD group (Table 4-3).

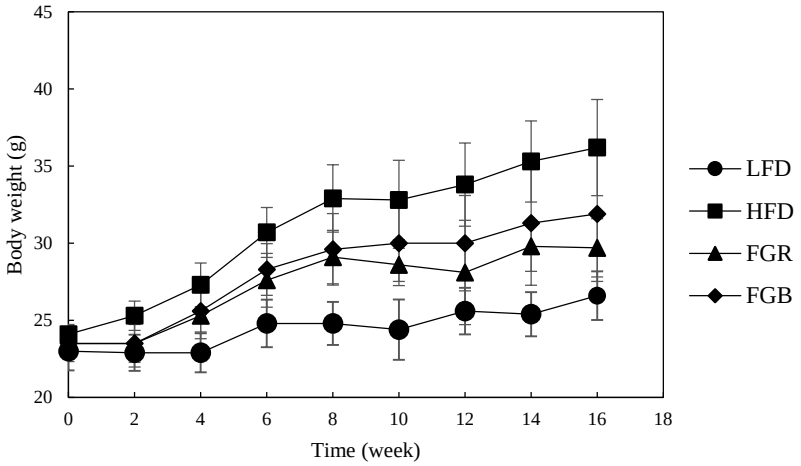


Fig. 4-1 Effects of crude saponins isolated from fermented ginseng root and berry on the body weight of mice fed a high-fat diet for 16 weeks (n=10).

Table 4-3 Effects of crude saponins isolated from fermented ginseng root and berry on the weight parameters of mice.

	LFD	HFD	FGR	FGB
Original BW (g)	23.0±1.2	24.2±0.6	23.3±1.2	23.3±1.0
Final BW (g)	26.1±1.6[a]	36.2±4.4[c]	29.7±1.9[b]	31.9±4.4[b]
Food intake (g)	299.0	311.2	260.1	248.4
FER (mg/g)	10.4	38.6	24.6	34.6
Liver (g)	1.0±0.2	1.1±0.3	0.9±0.2	1.0±0.2
Liver/BW (%)	4.0±0.7	3.3±0.8	3.2±0.6	3.2±0.3
EAT (g)	0.6±0.2[a]	1.8±0.6[b]	0.9±0.4[a]	1.6±0.6[b]
EAT/BW (%)	2.2±0.7[a]	5.2±1.3[b]	3.2±1.3[a]	5.0±1.2[b]

BW, body weight; FER, food effect ratio=(weight gain/food intake); EAT, epididymal adipose tissue;
[abc] Means not sharing a common letter are significantly different groups at $p<0.05$. (n=10)

4.3.2 Effects on blood glucose and lipid profiles

Mice in the FGR group had significantly lower levels of fasting blood glucose starting from week 12 while the FGB group showed only a sporadic slight hypoglycemic effect on blood glucose. At week 16, the fasting blood glucose levels were 89.8±7.7, 128.0±15.9, 94.5±8.2, 126.8±22.1 mg/dL, in the LFD, HFD, FGR and FGB group, respectively (Fig. 4-2). The results show that long-term consumption of HFD increases the fasting blood glucose levels of mice, and that supplementation of the root saponin significantly reduces the fasting blood glucose levels ($p<0.05$).

The mice in the FGR and FGB groups had similar levels of plasma TG and NEFA with the HFD group. For plasma cholesterol, the mice in both the FGR and FGB groups had significantly lower levels of LDL-C, and the mice in the FGR group also had significantly lower levels of TC and HDL-C compared with mice in the HFD group ($p<0.05$). HDL-C is considered as the “good cholesterol” and higher HDL-C levels are correlated with cardiovascular health. However, it is the ratio of HDL-C and LDL-C, rather than cholesterol itself, that matters [248]. In this study, both saponins from the root and berry slightly increased the ratio of HDL-C and LDL-C, though not statistically significantly (data not shown). Moreover, homeostasis model assessment of insulin resistance (HOMA-IR), a parameter of insulin resistance, was significantly lower in the FGR group ($p<0.05$). For reference, the ratio of adiponectin release versus epididymal adipose tissue

weight in the FGR group was remarkably higher than that in the HFD group (Table 4-4).

Table 4-4 Effects of crude saponins isolated from fermented ginseng root and berry on serological parameters of mice.

	LFD	HFD	FGR	FGB
TG (mg/dL)	64.9±9.2	55.0±12.1	56.7±18.3	56.5±2.6
TC (mg/dL)	77.3±12.4^{c}	136.3±9.0^{a}	113.3±7.2^{b}	128.2±11.6^{a}
LDL-C (mg/dL)	8.0±5.5^{c}	51.3±6.9^{a}	36.3±7.6^{b}	40.0±10.9^{b}
HDL-C (mg/dL)	58.1±8.4^{b}	75.5±6.8^{a}	57.3±14.6^{b}	74.7±3.9^{a}
NEFA (mEq/L)	1.5±0.2	1.5±0.2	1.7±0.2	1.6±0.3
Insulin (ng/mL)	1.7±0.8	1.9±0.8	1.3±0.1	1.7±0.2
HOMA-IR	11.2±8.0^{ab}	13.8±6.3^{a}	5.9±0.8^{b}	8.8±1.4^{ab}
Adiponectin (ng/mL)	4.0±1.0^{b}	5.5±0.9^{a}	5.8±0.5^{a}	4.4±0.6^{b}
ADP/EAT (ng/g)	6.7	3.1	6.4	2.8

^{abc} Means not sharing a common letter are significantly different groups at $p<0.05$ (n=6). ADP, adiponectin; EAT, epididymal adipose tissue. The homeostasis model assessment was used to calculate an index of insulin resistance (HOMA-IR) as insulin (mU/L) × glucose (mM)/22.5. NEFA, non-esterified fatty acid.

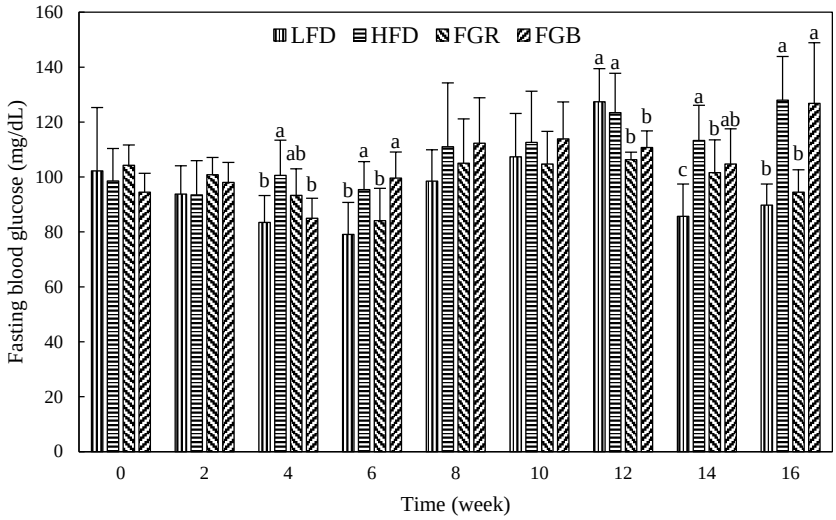


Fig. 4-2 Effects of FGR and FGB on fasting blood glucose of mice during the 16 weeks.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$ (n=10).

4.3.3 Effects on lipid metabolism in liver

Long term exposure to a HFD can cause NAFLD, a condition wherein large droplets of fat deposited in hepatocytes via the process of steatosis. Some studies have suggested that the kind of lipid rather than the amount of fat determines the susceptibility to the "second hit" of the "two-hit" theory for the pathogenesis of NAFLD [249]. Excessive cholesterol accumulation disrupts membrane fluidity, promotes cellular dysfunction, and thereby results in the progression of fatty liver [250].

In this study, the TG contents in liver were 291.9±42.3, 507.8±134, 270.8±68.6 and 252.6±79.3 mg/ g protein in the LFD, HFD, FGR and FGB group, respectively. Long-term exposure of HFD significantly increased the levels of liver TG contents while the root saponin and the berry saponin significantly reduced the TG contents ($p<0.05$, Fig. 4-3A, Fig. 4-4A). Moreover, the mice in both the FGR and FGB groups had markedly lower levels of hepatic TC contents ($p=0.096$, $p=0.007$, respectively). ALT, a liver injury marker, was significantly lower in the FGR group than in the HFD group (Fig. 4-4B, $p<0.05$). The expression of LDL-R, a receptor mediating the endocytosis of cholesterol-rich LDL, was significantly enhanced in the FGR and FGB group ($p<0.05$), which was in line with the decreased plasma LDL-C levels. HMG-CoA reductase, the rate-controlling enzyme in the pathway of cholesterol synthesis, also showed a decreased tendency in the FGR and FGB groups ($p=0.358$, $p=0.398$, respectively, Fig. 4-4C). As

described previously, cholesterol is used to neo-synthesize bile acids in a homeostatic response, resulting in a lower level of cholesterol in liver and plasma. It has been shown that red ginseng extract as well as ginsenosides can increase the expression of CYP7A1, CYP8B1 and MRP2 both *in vitro* and *in vivo* [116, 117], which might facilitates the synthesis of bile acids and biliary efflux from liver. In the present research, it could be interpreted that ginseng saponin might clear cholesterol in the blood via increasing the expression of LDL-R, and reduce cholesterol contents in the liver via inhibiting cholesterol synthesis, facilitating bile acid synthesis and biliary efflux (Fig. 4-5).

Obesity is accompanied by increased liver uptake of NEFAs which either undergo oxidation or are esterified with glycerol to produce TG. Recent studies shown that NEFA could directly cause toxicity by increasing oxidative stress and by activating inflammatory pathways, which aggravate fatty liver disease [251]. In this work, expressions of the gene Cd36 and Fatp5, mediating the liver uptake of NEFA, were repressed in the FGR and FGB groups. Especially, the mRNA level of Fatp5 in the FGR group was significantly lower than that in the HFD group ($p<0.05$). Low density lipoprotein receptor-related protein 1 (LRP1), mediating transfer of fat from chylomicron remnant to the liver, showed a decreasing trend in the FGR group ($p=0.15$). ApoB100, a protein used to assemble VLDL, showed an increasing trend in the FGR ($p=0.35$) and FGB ($p=0.09$) groups. Fatty acid-

binding protein 1 (FABP1) is involved in the transport and metabolism of long-chain fatty acids, and increased expression levels of gene Fabp1 have been observed in obese subjects, which is considered as a compensatory up-regulation in an attempt to counter the high metabolic stress associated with obesity [252]. The mRNA level of Fabp1 was reduced by 50% in the FGR group compared with the HFD group ($p=0.11$). Long-chain-fatty-acid CoA ligase 1 (ACSL1) plays a critical role in both fatty acid biosynthesis and β-oxidation [253]. Some reports have shown that HFD reduces Acsl1expression [254, 255] while others have shown that HFD induces Acsl1 expression at the mRNA level [256, 257]. In this study, the HFD did not significantly increase the mRNA levels of Acsl1 while FGR and FGB significantly decreased the expression levels of Acsl1 ($p<0.05$). Acyl-CoA-binding protein (ACBP), which mediates intermembrane acyl-CoA transport and donates acyl-CoA for β-oxidation or TG synthesis, showed an increased tendency in the FGR group ($p=0.15$, Fig. 4-4D).

Hepatic C/EBP-α plays a critical role in the acceleration of lipogenesis in ob/ob mice [258]. It was reported that PPAR-γ might be involved in HFD-induced liver steatosis [112]. The expression of these two factors was lower in the FGR group. The root saponin significantly inhibited the expression of C/EBP-α ($p<0.05$). In addition, expression of *Dgat2*, gene involved in TG synthesis, was significantly suppressed in the FGR group ($p<0.05$, Fig. 4-4E).

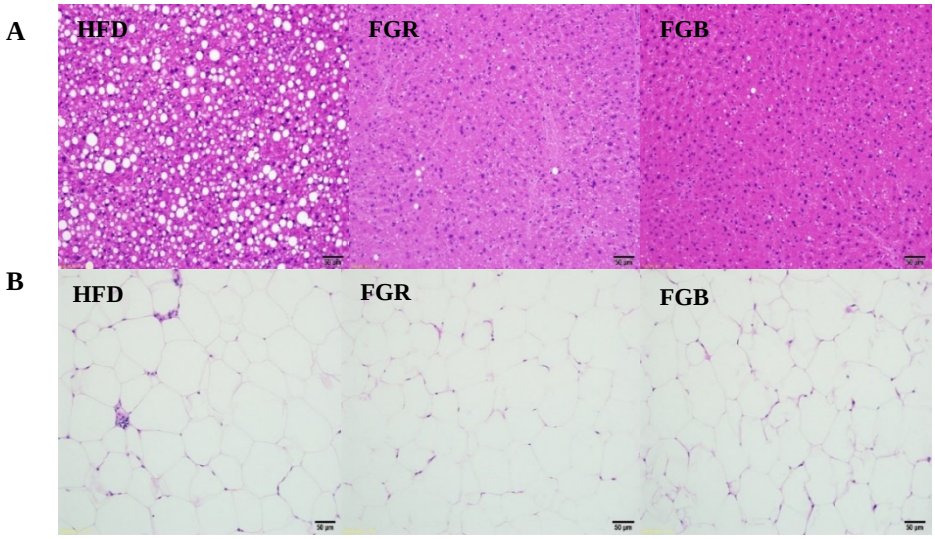


Fig. 4-3 The H&E staining of (A) liver and (B) epididymal adipose tissue in mice fed with high-fat diet for 16 weeks.

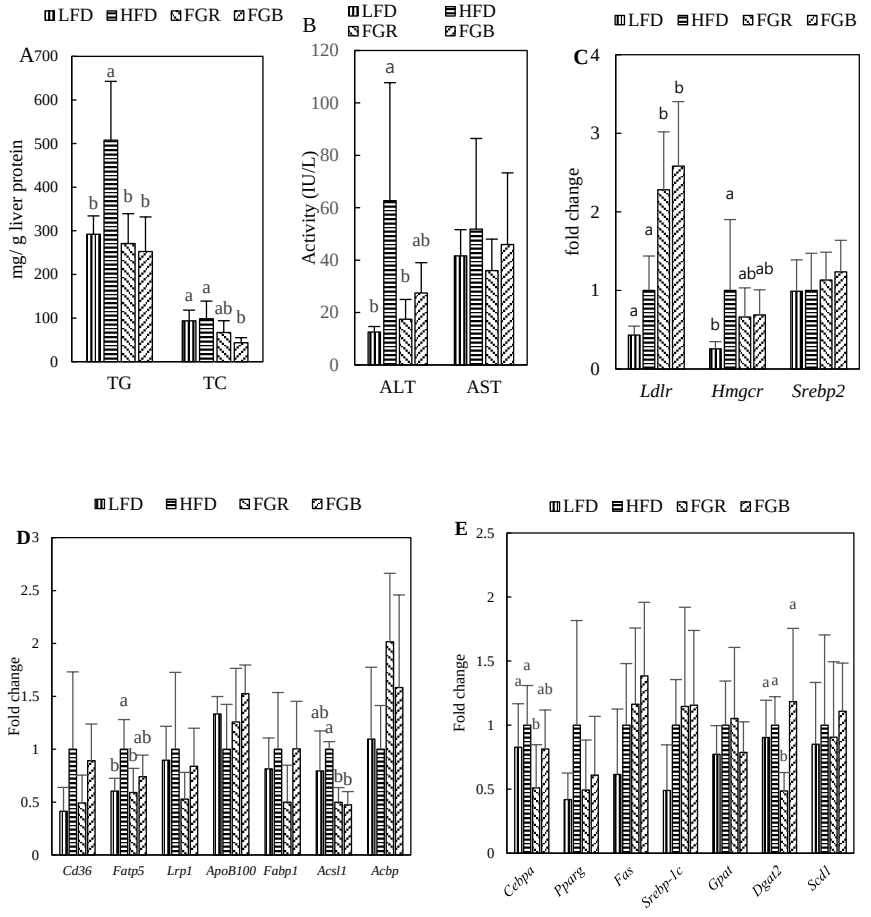


Fig. 4-4 Effects of crude saponins isolated from fermented ginseng root and berry in liver of mice fed a HFD for 16 weeks.

A, Effects on TG and TC levels in the liver (n=6); B, Effects on ALT and AST activities in the liver (n=6); C, Effects on gene expression related to cholesterol metabolism (n=4); D, Effects on gene expression related to fatty acid uptake and fatty acid channeling (n=4); E, Effects on gene expression related to fatty acid synthesis and lipogenesis (n=4). ^{abc} Means not sharing a common letter are significantly different groups at $p<0.05$.

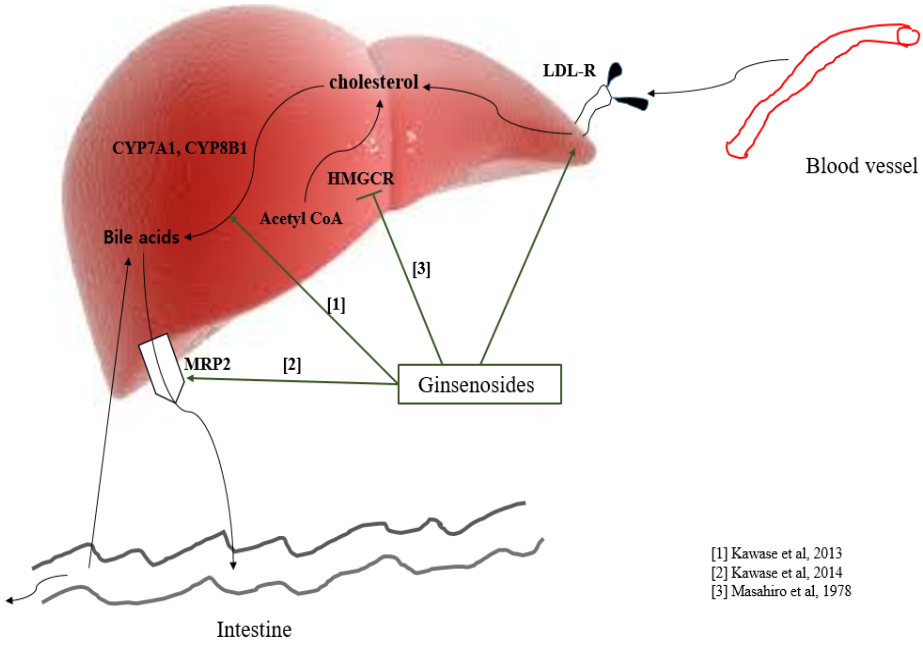


Fig. 4-5 Effects of ginseng or ginsenosides on the metabolism of cholesterol.

4.3.4 Effects of on the adipose tissue

The mice in the FGR group had a significantly lower weight of epididymal adipose tissue and smaller adipocyte size ($p<0.05$, Table 4-3, Fig. 4-3B, Fig. 4-6A). The mRNA expression levels of *Cd36* and *Lpl*, mediating the uptake of fatty acid, were significantly increased in the mice fed a HFD ($p<0.05$). FABP 4 is extensively used as a marker for differentiated adipocytes, and its blocking has the possibility of treating obesity [259]. The mRNA expression level of *Fabp4* was reduced by 38.2% ($p=0.188$) and 53.9% ($p=0.091$) in the FGR and FGB group, respectively. PPAR-γ and C/EBP-α, mediating adipogenesis in adipocytes [151], were down-regulated respectively by 35.9% ($p=0.241$) and 35.2% ($p=0.273$) in the FGR, and 76.6% ($p=0.019$) and 32.2% ($p=0.315$) in the FGB group. The mRNA level of *Fas* was significantly reduced by 67.1% in the FGB group ($p<0.05$). It was reported that the expression levels of *Atgl* and *Hsl* were both increased in the subcutaneous adipose tissue of HFD-induced obese mice, which was in line with this study [260]. The mRNA level of *Atgl* was reduced by 47.8% ($p=0.123$) in the FGB group and the mRNA level of *Hsl* was significantly reduced by 77.2% ($p<0.05$) in the FGB group compared with that in the HFD group (Fig. 4-6B).

Adipocyte hypoxia, due to adipose tissue hypertrophy resulting from obesity, can cause adipocyte necrosis, which leads to macrophage infiltration and secretion of pro-inflammatory cytokines [156]. It is reported

that TNF-α and IL-6 hamper the insulin cascade signaling pathway by preventing the phosphorylation of insulin receptors [261]. IL-1β also induces insulin resistance in adipocytes and its expression is upregulated in the adipose tissue of obese and insulin-resistant mice [262].

In this study, F4/80, used as macrophage markers, was significantly increased in mice fed a HFD and relatively lowered in the mice supplemented with root and berry saponins, especially with the berry saponin. Moreover, lower mRNA expression levels of cytokines were observed in the adipose tissue of mice in the FGR and FGB groups. The berry saponin supplementation significantly repressed the expression of IL-1β and IL-6 ($p<0.05$, Fig. 4-6C).

Taken together, the root saponin significantly decreased the epididymal fat weight of mice while the berry saponin significantly inhibited the mRNA expression of *Cebpa*, *Fas*, *Hsl*, *Il1b* and *Il6* in adipose tissue. W. Gu *et al*. [152] reported that ginsenoside Rh1 ameliorated HFD-induced obesity mice by inhibiting adipocyte differentiation and alleviated adipose inflammation. In fact, ginsenoside Rh1 is a metabolite of ginsenoside Re and is abundant in the berry saponin in this study.

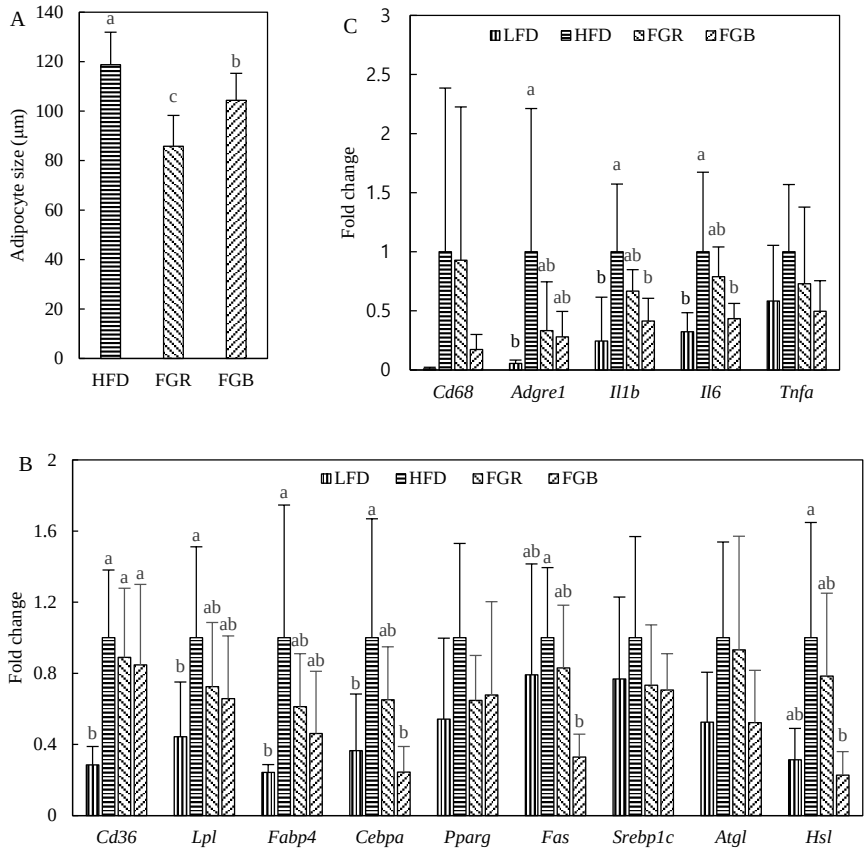


Fig. 4-6 Effects of crude saponins isolated from fermented ginseng root and berry in the adipose tissue of mice fed a HFD for 16 weeks.

A, adipocyte size. B. Effects on gene expression related to lipid metabolism (n=4); C, Effects on gene expression related to inflammation (n=4). ^{abc} Means not sharing a common letter are significantly different groups at $p<0.05$.

4.4 Summary

Crude saponins isolated from fermented ginseng root and berry significantly suppressed the weight gain of HFD-fed mice while only the root saponin potently attenuated hyperglycemia and insulin resistance. Both the root saponin and the berry saponin might improve hypercholesterolemia by facilitating expression of gene *Ldlr* and alleviate fatty liver through inhibiting liver uptake of free fatty acids. Only the berry saponin significantly inhibited inflammatory markers in adipose tissue. Overall, the root saponin showed a more potent anti-obesity effect on HFD-induced obese mice than the berry saponin.

Chapter 5 Anti-obesity effects of Ginsenoside compound K and Rh1 in obese mice induced with high fat diet

5.1 Introduction

As presented in chapter 4, crude saponins isolated from fermented ginseng root and fermented ginseng berry had significant anti-obesity effects in HFD-fed mice. In addition, ginseng root exerted a more potent activity than the berry. Kim *et al*. [81] reported that the PPD-type ginsenosides isolated from red ginseng had more potent anti-obesity activity than the PPT-type, which was in line with the present research. Now that the PPD-type and the PPT-type ginsenosides are mainly transformed to cK and Rh1, respectively, after oral ingestion, and that the root saponin contains abundant cK while berry saponin contains abundant Rh1. As described in Chapter 3, cK shows potent inhibitory effects on the activity of pancreatic lipase while Rh1 does not. It is reported that ginsenoside Rh1ameliorates HFD-induced obesity in mice by inhibiting adipocyte differentiation. However, the anti-obesity effects of purified cK in HFD-induced obese mice had not been reported yet. Whether cK exhibits more potent beneficial effects in HFD-induced obese mice than Rh1 remains to be elucidated. Moreover, whether cK and Rh1 are responsible for the anti-obesity effects of the root saponin and the berry saponin, respectively, needs to be confirmed.

The aim of this study is to evaluate and compare the anti-obesity effect of ginsenoside Rh1 with that of cK in obese ICR mice induced by HFD,

and confirm the responsible components for the activities of crude saponins isolated from fermented root and berry.

5.2 Materials and methods

5.2.1 Materials

Crude saponins isolated from FGR and FGB were same with the saponins sample in Chapter 4. Compound K and ginsenoside Rh1 were purchased from Biotech (Nanjing, China) and Cogon Biotech (Chengdu, China), respectively. Orlistat was purchased from Sigma (St. Louis, MO).

5.2.2 Animal and diets

Male ICR mice (5 weeks old), purchased from Central Lab. Animal (Seoul, Korea), were housed under a 12 h light/12 h dark cycle in a controlled room at a temperature of 23 ± 3°C and a humidity of 50% ± 10%. After acclimating to the facility for 1 week, the mice were randomly divided into 7 groups (n = 9) and each named LFD, HFD, FGR, FGB, cK, Rh1 and orlistat, respectively. Mice in LFD group were fed a low fat diet (LFD; 10% of the total calories from fat, Table 5-1), and mice in other groups were fed a HFD (60% of the total calories from fat, Table 5-1). All the mice were allowed food and water *ad libitum*. After 4 weeks, mice in the FGR and FGB groups were respectively administered 150 mg/kg/d, and mice in the cK, Rh1 and orlistat groups were respectively administered 30 mg/kg/d cK, Rh1 and orlistat by gavage for another 4 weeks. Body weight was determined once a week and food intake was determined once every 2 weeks. Then all the mice underwent 12 h of fasting prior to being

anaesthetized with zoletil™ 50 (Virbac, Carros, France) and rompun® (Ansan, Korea) and then were dissected. Blood samples were collected by heart punctures. Epididymal fat pads were removed and stored at −80°C for subsequent analyses. All procedures relating to the animals and their care were approved by the Institutional Animal Care and Use Committee of Seoul National University.

Table 5-1 Formula of low fat diet and high fat diet.

Formula	LFD (10% calorie from fat) g/Kg	HFD (60% calorie from fat) g/Kg
Casein	210.0	265.0
L-Cystine	3.0	4.0
Corn Starch	280.0	0
Maltodextrin	50.0	160.0
Sucrose	325.0	90.0
Lard	20.0	310.0
Soybean Oil	20.0	30.0
Cellulose	37.15	65.5
Mineral Mix, AIN-93G-MX (94046)	35.0	48.0
Calcium Phosphate, dibasic	2.0	3.4
Vitamin Mix, AIN-93-VX (94047)	15.0	21.0
Choline Bitartrate	2.75	3.0
Yellow Food Color	0.1	0
Blue Food Color	0	0.1

5.2.3 Histopathologic evaluation

After mice were sacrificed, the samples of epididymal fat pads were fixed with formalin solution, stained with hematoxylin and eosin, and viewed with an optical microscope.

5.2.4 Real-time polymerase chain reaction

Total RNA was extracted from the adipose tissue with an RNeasy® Lipid Tissue Mini Kit from Qiagen (Venlo, Netherlands), respectively. The concentration of RNA was measured with the Micro Spectrophotometer from Allsheng (Hangzhou, China), and 0.5 μg of total RNA from each sample was reverse-transcribed to cDNA by with a cDNA synthesis kit from Takara Bio (Kusatsu, Japan). Relative quantifications of gene transcripts were completed with SYBR premix from Takara Bio using the Applied Biosystems 7500 system. Relative mRNA levels were normalized to the *Gapdh* mRNA level and expressed as values of relative expression compared to that of the HFD group.

5.2.5 Statistical analysis

The differences among groups were examined with one-way ANOVA followed by the Least significant range tests. Statistical analyses were done with the SPSS statistical package (Chicago, IL). The significance level of the test results was set at $p < 0.05$.

5.3 Results

5.3.1 Calorie intake and body weight

Mice fed on a low-fat diet showed significantly lower calorie intake than mice fed on a high-fat diet (Fig. 5-1). However, administration of the root saponin, berry saponin and cK almost reversed the calorie intake of high-fat diet fed mice to a normal level. In special, the root saponin significantly inhibited calorie intake of mice in week 5-6 and cK significantly inhibited the calorie intake of mice in week 7-8 ($p<0.05$, Fig. 5-1 and Fig. 5-2).

Mice in the HFD group had significantly higher body weight from week 2 than mice in the LFD group ($p<0.05$). From week 5, mice were respectively treated with the root saponin, berry saponin, cK, ginsenoside Rh1 and orlistat. The experimental groups had relatively lower body weight compared with the HFD group (Fig 5-3). After treatment, the body weight of mice were respectively 46.6 ± 1.7, 62.1 ± 7.6, 52.5 ± 6.2, 57.8 ± 8.0, 53.8 ± 8.3, 58.9 ± 9.5 and 54.9 ± 8.9 g in the LFD, HFD, FGR, FGB, cK, Rh1 and orlistat group. Mice in the FGR and cK groups had significantly lower body weight from week 7 than mice in the HFD group ($p<0.05$). As shown in Fig. 5-4, the body weight gain of before and after treatment in FGR, FGB, cK and orlistat group, respectively 4.0 ± 2.8, 6.1 ± 4.1, 4.3 ± 2.4, 4.6 ± 3.1 g, was significantly lower than that in the HFD group (10.9 ± 2.4 g, $p<0.05$).

Although the body weight gain in the Rh1 group (8.6±2.6 g) was relatively lower than that in the HFD group, the result is not significant ($p=0.116$). Food effect ratio, a parameter representing the body weight gain per unit food intake, was 26.1±2.9, 89.5±14.8, 36.2±25.9, 58.8±13.0, 41.4±15.0, 69.5±6.9, 40.7±21.2 mg/g in the LFD, HFD, FGR, FGB, cK, Rh1 and orlistat group, respectively (Fig. 5-5). Mice in the FGR, FGB, cK and orlistat group had significantly lower food efficiency ($p<0.05$). Ginsenoside Rh1 failed to significantly reduce food efficiency ($p=0.147$).

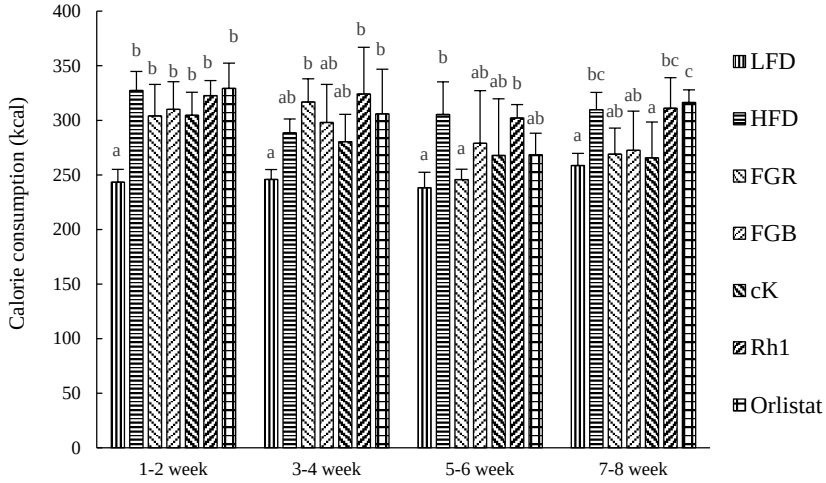


Fig. 5-1 Average calorie intake of mice determined every 2 weeks.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$.

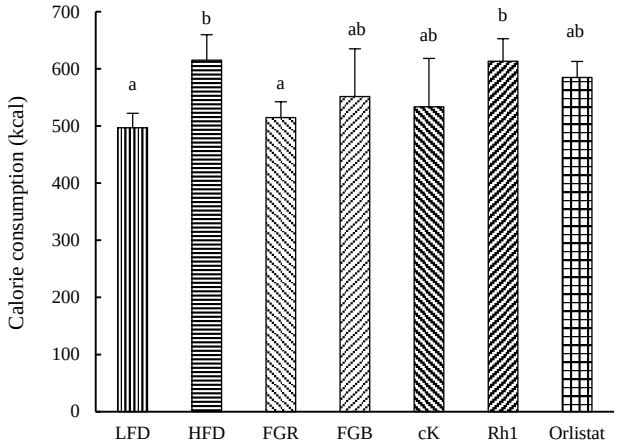


Fig. 5-2 Total calorie consumption of mice treated with crude saponins isolated from fermented ginseng root and berry, as well as ginsenoside cK, Rh1 and orlistat for 4 weeks.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$.

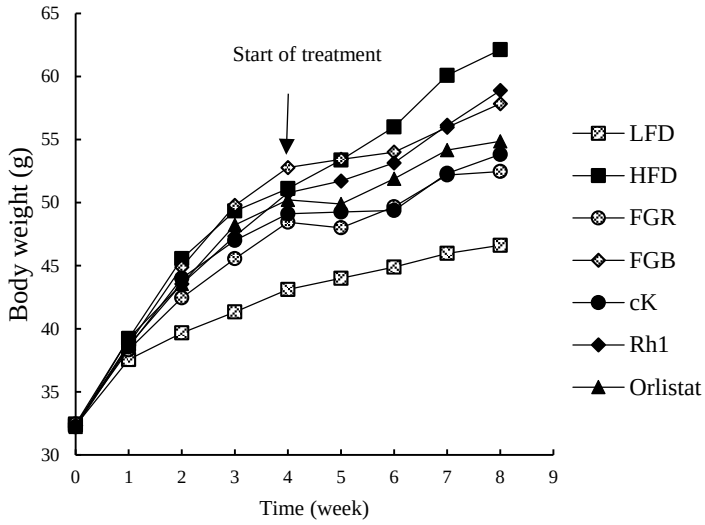


Fig. 5-3 Effects of crude saponins from fermented ginseng root and berry as well as ginsenoside cK, Rh1 and orlistat on the body weight of mice (n=9).

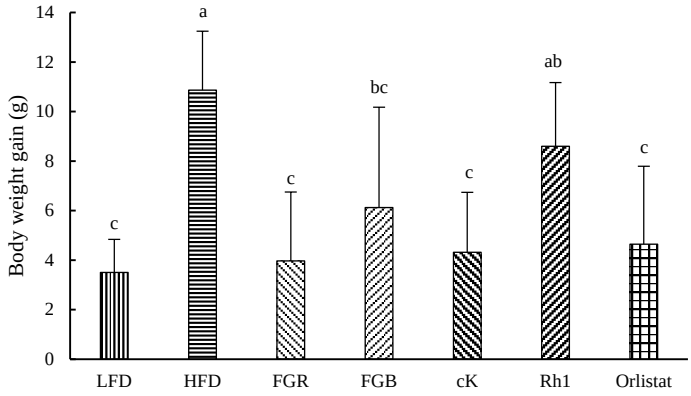


Fig. 5-4 Body weight gain of mice treated with crude saponins isolated from fermented ginseng root and berry, as well as ginsenoside cK, Rh1 and orlistat for 4 weeks.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$ (n=9).

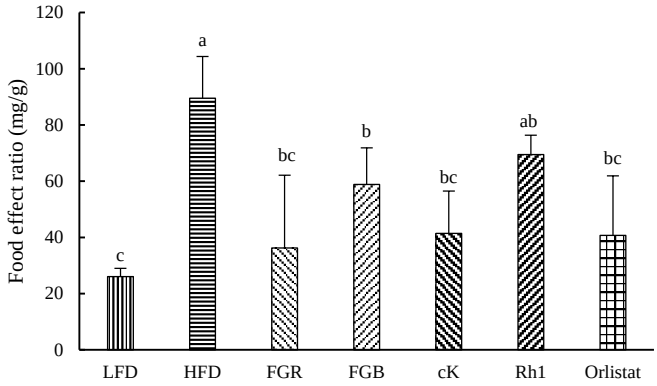


Fig. 5-5 Food effect ratio of mice treated with crude saponins isolated from fermented ginseng root and berry, as well as ginsenoside cK, Rh1 and orlistat for 4 weeks.

Food effect ratio (FER) = body weight gain (mg) / food intake (g). ^{abc} Means not sharing a common letter are significantly different groups at $p<0.05$.

5.3.2 Fat deposition and adipocyte size

After treated for 4 weeks, mice were sacrificed and the epididymal fat, mesenteric fat and perirenal fat were removed and weighted (Fig. 5-6). Mice fed on a low-fat diet had significantly smaller epididymal fat, mesenteric fat and perirenal fat tissues. The epididymal fat of mice in FGR, Rh1 and orlistat groups was significantly lower than that in HFD group ($p<0.05$). The weight of mesenteric fat and perirenal fat in FGR, cK, orlistat groups were significantly lower than that in HFD groups ($p<0.05$). Mice in the Rh1 group also had significantly lower mesenteric fat tissue compare with mice in the HFD group ($p<0.05$). The adipocyte size in was 117.2 ± 12.2, 130.3 ± 12.7, 117.2 ± 14.9, 124.7 ± 13.4, 120.9 ± 14.8, 124.9 ± 18.1 and 125.9 ± 10.8 μm in the LFD, HFD, FGR, FGB, cK, Rh1 and orlistat group, respectively. The adipocyte size of mice in FGR group was significantly lower than that in the HFD group and was reversed to the normal level ($p<0.05$, Fig. 5-7 and Fig. 5-8).

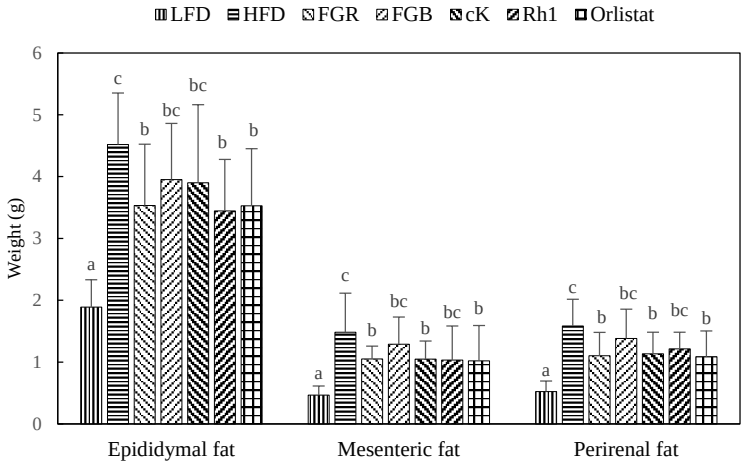


Fig. 5-6 The weight of epididymal fat, mesenteric fat and perirenal fat of mice treated with crude saponins isolated from fermented ginseng root or berry, as well as ginsenoside cK, Rh1 and orlistat for 4 weeks.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$ (n=9).

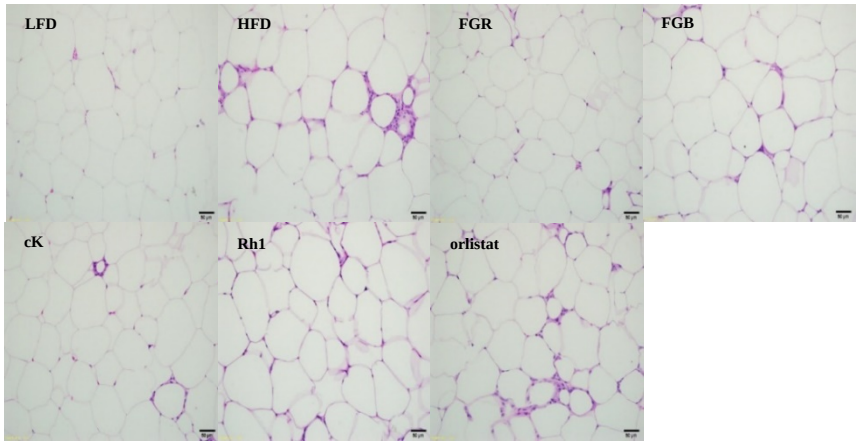


Fig. 5-7 The H&E staining of epididymal adipose tissue of mice after treatment with crude saponins isolated from fermented ginseng root or berry, as well as ginsenoside cK, Rh1 and orlistat for 4 weeks.

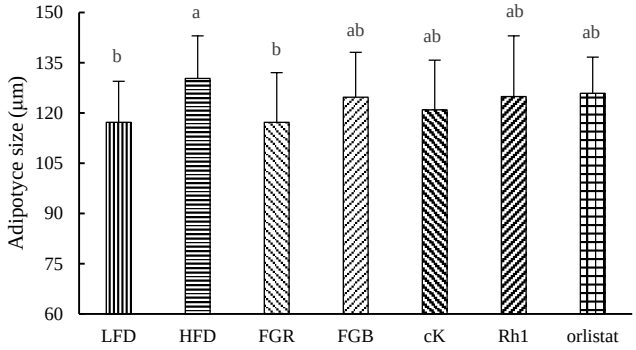


Fig. 5-8 The adipocyte size of mice after treatment with crude saponins isolated from fermented ginseng root or berry, as well as ginsenoside cK, Rh1 and orlistat for 4 weeks.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$.

5.3.3 The expression of lipogenesis-related gene in adipose tissue

The mRNA expression levels of gene *Srebp-1c* was significantly reduced by 69.4% in cK group ($p<0.05$, Fig. 5-9A). The mRNA expression levels of gene *Acc* were significantly reduced by 32.9%, 54.8% and 34.1% in FGR, FGB and orlistat groups, respectively, when compare with the HFD group (Fig. 5-9B). In addition, expression of gene *Fas* was reduced by 82.2%, 86.8%, 65.6% and 73.6% in FGR, FGB, cK and orlistat group, respectively ($p<0.05$, Fig. 5-9C).

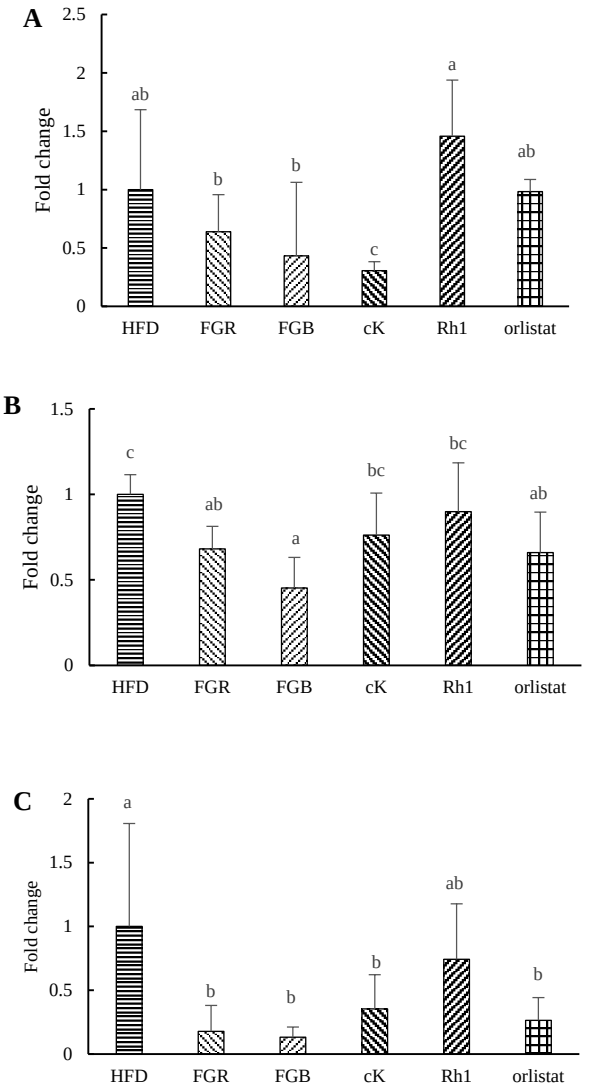


Fig. 5-9 Effects of crude saponins isolated from fermented ginseng root or berry, as well as ginsenoside cK, Rh1 and orlistat on mRNA levels of lipogenesis-related genes in adipose tissue.

[abc] Means not sharing a common letter are significantly different groups at $p<0.05$.

5.4 Discussion

As described in Chapter 1, ginsenosides, especially the PPD-type, can improve appetite regulation disorder induced by chronic inflammation in the hypothalamus due to long term consumption of high-fat diet [247]. In this work, cK and the root saponin significantly showed an anorexigenic effect and almost reversed the calorie intake of mice to the normal level. Mice treated with the berry saponin also had relatively low levels of calorie intake while ginsenoside Rh1 and orlistat showed no effect. Moreover, only mice treated with cK and the root saponin had significantly lower body weight. Compound K, root saponin, berry saponin and orlistat significantly inhibited body weight gain.

As mentioned, cK, orlistat, and saponins from root or berry can inhibit the activity of pancreatic lipase that catalyzes fat digestion in the intestine. Especially, the main mechanism of orlistat, a commercial diet pill, is that it targets the pancreatic lipase. As observed in Chapter 3, the feces of mice treated with the root saponin contained more triglyceride than that of mice in the control group, which indicates that the root saponin might suppress the digestion and utilization of triglyceride.

Mice treated with the root saponin, berry saponin, cK and orlistat had significantly lower food effect ratio. The food effect ratio in the FGB group, however, was not significantly reduced in Chapter 4. Moreover, mice in

FGB group had relatively lower levels of food intake when compared with mice in the FGR group, which is inconsistent with this study. The saponins were mixed with diets in Chapter 4, whereas mice were administered by gavage in this study. The saponins mixed with diet may alter the flavor or odor, and thereby affect the appetite of mice, which might be the reason for these contradictions.

With respect to fat deposition in the adipose tissue, the root saponin and cK had similar inhibitory effects, which were also similar with the effect of orlistat. Contrary to the berry saponin, ginsenoside Rh1 also significantly reduced fat storage of mice (Fig. 5-6). In addition, only the root saponin-treated mice had significantly reduced the adipocyte size (Fig. 5-8). From the photos of H&E staining of the adipose tissue, it could be found that mice treated with the root saponin, berry saponin, cK and ginsenoside Rh1 had lower levels of “crown structures”.

The ACC is an enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA, which plays a key role in chain elongation of fatty acid biosynthesis. Mammalian ACC is regulated transcriptionally by multiple promoters which mediates ACC abundance in response to the cell nutritional status [263]. The sensitivity to nutritional status results from the control of these promoters by transcription factors such as SREBP-1c, which regulates genes required for glucose metabolism and fatty acid and lipid production [264]. The ACC also can be regulated

by the phosphorylation via AMPK due to a high level of AMP when energy status is low. The phosphorylated ACC switch lipogenesis toβ-oxidation. In this study, treatment with the root saponin, berry saponin and orlistat significantly down-regulated the expression of gene *Acc*. Only cK significantly reduced the expression of gene *Srebp-1c*. Except ginsenoside Rh1, all the experimental groups had significantly lower expression levels of gene *Fas* when compared with the HFD group.

Overall, cK showed a similar pattern of effect with the root saponin. However, ginsenoside Rh1 and the berry saponin showed a different pattern of effect. As the berry extract also contains the PPD-type ginsenosides and other chemicals such as polysaccharide, vitamin E, flavonoid, etc. there might be other ginsenosides and other active compounds which are responsible for the anti-obesity activity of the crude saponin isolated from ginseng berry.

5.5 Summary

Crude saponin isolated from fermented ginseng root significantly reduced calorie intake, fat digestion, body weight, body weight gain, food effect ratio, fat deposition and adipocyte size, and down-regulated the expression of gene *Acc* and *Fas* in the adipose tissue of mice. Ginsenoside cK significantly reduced calorie intake, body weight, body weight gain, food effect ratio, and fat deposition, and down-regulated the expression of gene *Srebp-1c* and *Fas* in the adipose tissue of mice. Orlistat significantly reduced body weight gain, food effect ratio and fat deposition, and down-regulated the expression of gene *Acc* and *Fas* in the adipose tissue of mice. The root saponin and cK showed the similar anti-obesity effects, which indicates that cK might be mainly principle for the activities of fermented ginseng root. Moreover, the anti-obesity effects of the root saponin and cK was as potent as orlistat. Crude saponin isolated from fermented ginseng berry significantly reduced body weight gain, food effect ratio, and down-regulated the expression of gene *Acc* and *Fas* in the adipose tissue of mice while ginsenoside Rh1 only significantly reduced fat deposition, which indicates that there might be other ginsenosides or other active compounds responsible for the anti-obesity effect of fermented ginseng berry.

Overall, cK and crude saponin isolated from fermented ginseng root or berry might exert potent anti-obesity effects in obese mice induced by high-

fat diet through preventing excess food intake, reducing fat over-absorption and inhibiting lipogenesis in the adipose tissue. Ginsenoside cK might be one of the main components which are responsible for the protective effect of the crude saponin isolated from fermented ginseng root against obesity. In addition, cK is more potent than Rh1 and the root saponin is more potent than the berry saponin considering the anti-obesity activity in high-fat diet induced obese mice, which is consistent with the results in Chapter 4.

Chapter 6 Summary and conclusion

Ginseng is a traditional tonic and a widely used functional food. Various compounds including ginsenoside, polysaccharide, oliopeptide and polyacetylene are considered responsible for the bioactivities of ginseng. Among these compounds, ginsenosides are attracting increasing attentions and have been actively researched for half a century. Effects of ginsenosides on the central nervous system, the cardiovascular system, and the immune system have been reported.

It is reported that ginseng and ginsenosides might have potential anti-obesity effects. Ginseng's effects on food intake, the digestion and absorption systems, as well as in the liver, adipose tissue, and skeletal muscle are evaluated in order to identify the mechanisms involved. Our review of previous *in vitro* and *in vivo* studies revealed that ginseng and ginsenosides could increase energy expenditure by stimulating the AMPK pathway and reduce energy consumption.

As the deglycosylated ginsenosides can be absorbed more easily in the intestinal tract, it is necessary to transform ginsenosides before oral ingestion. Various strains of *A. niger* and *A. orzyae* were screened in order to transform ginsenosides. It is found that *A. niger* is more inclined to transform the PPD-type ginsenoside to compound K, and that *A. orzyae* is more inclined to transform the PPT-type ginsenoside to Rh1. Ginseng root and ginseng berry have distinct ginsenoside profiles. The main ginsenoside in the root is the PPD-type, like Rb1, while the main ginsenoside in the

berry is the PPT-type, like Re. Therefore, *A. niger* FMB S494 and *A. oryzae* FMB S247 were selected to ferment ginseng root and ginseng berry, respectively. These two strains do not produce mycotoxin and can be considered safe. After fermentation, *A. niger* FMB S494 effectively transformed the PPD-type ginsenosides in the root, with a high yield of compound K (9.1%), while *A. oryzae* FMB 40247 effectively transformed the PPT-type ginsenosides in the berry, with a high yield of Rg1 (19.1%) and Rh1 (7.8%).

Retarding the activity of pancreatic lipase in the intestinal tract reduces energy harvest, which help to prevent and improve obesity. The effects of various ginsenosides and crude saponins isolated from ginseng root and berry on the activity of pancreatic lipase were observed. The results showed that the effects vary with each individual ginsenoside. Ginsenoside Rb1, Rd, Rg1, Rg3, and cK significantly suppressed 43%, 47%, 75% and 55% of lipase activity in vitro at the concentration of 100 μg/mL, respectively. Rg3 is discovered to be the most effective among various common ginsenosides, with a minimum effective concentration of 6.25μg/mL. The PPD-type ginsenosides are more potent than the PPT-type. In addition, fermentation dramatically enhances the inhibitory effect of the crude saponin isolated from ginseng root and ginseng berry, which might be attributed to the changes of ginsenoside profiles. Moreover, the root saponin shows more potent inhibitory effect than the berry saponin, which might be due to the

higher PPD-type ginsenoside content in the root.

Previous studies have shown that both the root and the berry exhibit anti-obesity and anti-diabetic effects. However, a direct comparison of the protective effects against obesity in high-fat diet-induced obese mice between the root saponin and the berry saponin after oral administration remains to be illuminated. The present research show that both the root and the berry saponins significantly suppress weight gain and excess energy consumption and improve hypercholesterolemia and fatty liver while only the root saponin significantly attenuates hyperglycemia and insulin resistance. Both the root saponin and the berry saponin significantly inhibit the mRNA expression of gene *Ldlr* and *Acsl1* while only the root saponin significantly inhibits the expression of *Cebpa* and *Dgat2* in liver. Moreover, the root saponin significantly decreases the epididymal fat weight of mice while the berry saponin significantly inhibits the mRNA expression of gene *Cebpa*, *Fas*, *Hsl*, *Il1b* and *Il6* in adipose tissue. Both saponins from the root and the berry have a beneficial effect on HFD-induced obesity. Compared to the berry saponin, the root saponin exhibited more potent anti-hyperglycemic and anti-obesity effect. However, only the berry saponin significantly inhibits the mRNA expression of inflammatory markers such as IL-1β and IL-6 in adipose tissue.

Compound K and ginsenoside Rh1 are the main metabolite of the PPD-type and the PPT-type ginsenosides, respectively, and can be absorbed

through the enterocyte directly. In order to confirm whether cK and Rh1 are respectively responsible for the anti-obesity effect of the root saponin and the berry saponin, the protective effects of cK and Rh1, as well as the root saponin and the berry saponin against obesity in high-fat diet induced obese ICR mice were observed.

The root saponin significantly reduces calorie intake, body weight, body weight gain, food effect ratio, fat deposition and adipocyte size, and down-regulates the expression of gene *Acc* and *Fas* in the adipose tissue. Ginsenoside cK significantly reduces calorie intake, body weight, body weight gain, food effect ratio, and fat deposition, and down-regulates the expression of gene *Srebp-1c* and *Fas* in the adipose tissue. The root saponin and cK show the similar anti-obesity effects, which indicates that cK might be responsible for the activities of fermented ginseng root. Moreover, the anti-obesity effects of the root saponin and cK are as potent as orlistat.

The berry saponin significantly reduces body weight gain, food effect ratio, and down-regulates the expression of gene *Acc* and *Fas* in the adipose tissue while Rh1 only significantly reduced fat deposition, which indicates that there might be other ginsenosides or other active compounds responsible for the anti-obesity effect of fermented ginseng berry.

Despite the different way of administration, different germline of mice and different length of experiment duration, the effects of the root saponin

and the berry saponin on body weight gain and food intake in Chapter 4 and Chapter 5 are consistent with each other.

The highlights of the present research are listed as follow:

1) *A. niger* intents to transform the PPD-type ginsenosides while *A. orzyae* intends to transform the PPT-type ginsenoside.

2) *A. niger* FMB S494 potently transformed the PPD-type ginsenoside like Rb1, Rb2, Rd to cK while *A. orzyae* FMB S247 potently transformed the PPT-type ginsenoside like Re, Rg1 to Rh1.

3) The transformed ginsenoside, Rg3 and cK, more potently suppress the activity of pancreatic lipase.

4) Fermentation dramatically enhances the inhibitory effect of crude saponin isolated from ginseng root or ginseng berry on the activity of pancreatic lipase.

5) The PPD-type ginsenoside more potently suppresses the activity of pancreatic lipase than the PPT-type.

6) The root saponin more potently suppresses the activity of pancreatic lipase than the berry saponin.

7) Both the root saponin and the berry saponin significantly suppress weight gain and excess food intake, and improve hypercholesterolemia and fatty liver while only the root saponin significantly attenuates

hyperglycemia and insulin resistance in HFD-fed C57BL/6 mice.

8) Compared to the berry saponin, the root saponin exhibits more potent anti-hyperglycemic and anti-obesity effect. However, only the berry saponin significantly inhibits mRNA expression of inflammatory markers such as IL-1β and IL-6 in the adipose tissue.

9) Ginsenoside cK significantly reduces calorie intake, body weight, body weight gain, food effect ratio, and fat deposition, and down-regulates the expression of gene *Srebp-1c* and *Fas* in the adipose tissue of mice.

10) Ginsenoside Rh1 only reduces fat deposition and slightly suppresses body weight gain in HFD-induced obese mice.

11) Ginsenoside cK shows more potent beneficial effects in obese mice induced with HFD than Rh1.

In conclusion, ginsenoside cK and crude saponin isolated from fermented ginseng root or fermented ginseng berry might exert potent anti-obesity effects in obese mice induced by high-fat diet via preventing excess food intake, reducing fat over-absorption and inhibiting lipogenesis in the adipose tissue. Ginsenoside cK is more potent than Rh1 and the root saponin is more potent than the berry saponin considering the inhibitory effect on the activity of pancreatic lipase and the protective activity against obesity in high-fat diet induced obese mice. These findings might provide guiding significance for application of ginseng root and ginseng berry on

the protection against obesity.

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국문초록

인삼은 다양한 생리학적 기능이 있으며, 수 천년 동안 약초로 사용되어왔다. 인삼과 진세노사이드는 AMPK 경로를 자극함으로써 에너지 소비를 증가시키고 과다한 에너지 섭취를 억제할 수 있다. 인삼 뿌리의 대표적인 사포닌은 PPD 형이며, 인삼 열매에서는 PPT 형이다. 이처럼 뿌리와 열매가 함유하는 진세노사이드가 다르기 때문에, 서로 다른 효능을 가질 수 있다. 탈글리코실화 된 진세노사이드가 주요 흡수 형태이기 때문에, 경구 섭취 전에 진세노사이드를 변형시킬 필요성이 제기되고 있다. 또한, 소장에서 지방의 소화 흡수를 억제하면 에너지 수확을 줄여 비만 예방 및 개선에 도움이 된다. 본 연구는 다양한 곰팡이로 인삼 뿌리와 열매를 발효하여, 췌장 리파제 (pancreatic lipase, PL) 활성의 억제, 체중과 지질대사 조절 등 측면에서 뿌리와 열매 사포닌의 항 비만 효능을 비교하였다. 연구결과는 *A. niger*가 PPD 형 진세노사이드를 cK로 변형시키며, *A. orzyae*가 PPT 형 진세노사이드를 Rh1로 변형시킨다. 진균독을 생산하지 않는 *A. niger* FMB S494와 *A. orzyae* FMB S247로 발효한 인삼 뿌리와 열매는 각각 풍부한 cK 및 Rh1을 함유한다. PL 활성 분석의 결과에 따르면, PPD 형 진세노사이드가 PPT 형보다 더 강력한 억제 효과를 갖는다. 더욱이, 발효가 인삼 뿌리와 열매 사포닌의 억제 능력을 현저히 강화시켰다. 또한 뿌리 사포닌 경구투여 시 고지방식이의 쥐의 변에서 중성지방의 함량이 유의하게 높았다. 따라서 뿌리 사포닌이 in vitro와 in vivo에서 열매 사포닌보다 PL 활성에 더 강력한 억제 효력을 있음을 알 수 있다. 동물 실험의 결과에 따르면 두 개의 사포닌이 모두 체중 증가를 유의하게 억제하고 고지혈증 및 지방간을 개선하는 반면, 뿌리 사포닌

만이 고혈당증 및 인슐린 저항성을 유의하게 개선시키는 것으로 나타났다. 뿌리 사포닌과 열매 사포닌은 HFD로 유도된 비만에 유익한 효과가 있었다. 열매 사포닌과 비교하면, 뿌리 사포닌은 보다 강력한 항 고혈당 및 항 비만 효과를 나타냈다. 그러나, 열매 사포닌만이 지방 조직에서 IL-1β 및 IL-6 등 염증 마커의 mRNA 발현을 유의하게 억제하였다. cK와 Rh1은 각각 PPD 형과 PPT 형 인삼 사포닌의 흡수 형태이기 때문에 뿌리 사포닌과 베리 사포닌의 항 비만 효과를 담당하는지의 여부를 확인하였다. 뿌리 사포닌과 cK는 과도한 칼로리 섭취, 체중 증가, 식이효율, 지방 침착을 유의하게 줄이고 지방 조직에서 유전자 Fas의 발현을 하향 조정하였다. 따라서 cK가 발효 인삼 뿌리의 비만 억제 작용을 담당한다고 결론 지을 수 있다. 열매 사포닌은 체지방 증가, 식이효율을 미약하게 감소시키고 지방 조직에서 유전자 Fas의 발현을 낮추어 주는 반면 Rh1은 지방 침착만 감소시켰다. 이는 다른 인삼 사포닌이나 활성 물질도 인삼 열매의 항 비만 효과에 기여할 것을 설명한다. 결론적으로, PL 활성, 과량의 식이 섭취 및 체중 증가에 대한 억제효과를 고려하여, cK 및 뿌리 사포닌은 Rh1 및 열매 사포닌보다 더 강력한 항 비만 효과를 각각 나타낸다.

주요어: 인삼 뿌리; 인삼 열매; 췌장 리파제; 고지방 식이; 비만

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